VARIATIONS IN SIZE OF CORPORA LUTEA IN THE ALBINO RAT UNDER NORMAL AND EXPERIMENTAL CONDITIONS [1]

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ONE FIGURE

Since the pioneer work of Long and Evans ('22) much emphasis has been placed upon the size of corpora lutea in rats under various conditions. The most striking observation has been that during the latter half of pregnancy the corpora lutea are larger than at any other time. Long and Evans distinguished between four kinds of corpora lutea found in the rat ovary at different times, viz., those of ovulation, pseudopregnancy, pregnancy and lactation. They pointed out that the maximum diameter of the corpora lutea of ovulation is 1.2 mm.; of pseudopregnancy, 1.2 to 1.4 mm.; of pregnancy, about 2.0 mm. and of lactation, 1.2 mm. Swezy ('33) reported a few measurements of corpora lutea which, for the most part, agree with those of Long and Evans. She pointed out, as did the latter investigators, that the corpora lutea of pregnancy cannot be distinguished from those of ovulation until approximately the tenth day of gestation. Between the tenth and fourteenth days the corpora lutea grow to the maximum diameter of about 2.0 mm., and remain at this size until the end of pregnancy. Her measurements

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also showed that the corpora lutea of pseudopregnancy are slightly larger than those of ovulation but considerably smaller than those of the latter half of pregnancy. Weichert ('40) has reported that in delayed pregnancy [2] the size of the corpora lutea in pregnant lactating rats on the twenty-first day of gestation is inversely correlated with the number of suckling young.

Except for these data, we have found only a few incomplete reports giving accurate measurements of corpus luteum for the rat. Since the size of corpora lutea is often referred to in endocrine research, we feel that a clarification of such vague terms as large, small or medium sized with reference to corpora lutea would be helpful. We are, therefore, presenting a more complete set of data with special emphasis on the sizes of corpora lutea during normal pregnancy, delayed pregnancy and following the administration of estrogens, prolactin and Antuitrin-S.

METHODS

The measurements of corpora lutea reported by Long and Evans ('22) and Swezy ('33) are described as "longest diameter" measurements. After examining a large number of ovaries the authors have found that the longest diameters of corpora lutea often vary considerably in any particular ovary. They feel, therefore, that a more accurate result may be obtained by measuring two opposite diameters of each corpus luteum and taking an average. In obtaining the data presented here, each of the two diameters was measured twice and the resulting four readings were averaged to procure a value for each corpus luteum. The data for all corpora lutea in each rat were then averaged and the resulting figure was

[2] It has been known for some time that the gestation periods of pregnant lactating rats and mice may be unduly prolonged due to a delay in implantation. According to some investigators the delay is correlated in general with the number of suckling young (Daniel, '10; King, '13; Enzmann, Saphir and Pincus, '32; Weichert, '40, '41, '42). Others are of the opinion that the number of suckling young bears but little relation to the delay in implantation (Kirkham, '18; Hain, '34 and Krehbiel, '41).

taken as typical for the particular animal in question. As many corpora lutea were measured as remained in both ovaries after removal of a few (usually four) for histological study.

The ovaries were fixed in Bouin's solution and run up to 80% alcohol before being measured. Each ovary was placed in a dish of 80% alcohol, then for a few seconds on an absorbent towel and measured with a Vernier caliper under a binocular dissecting microscope. It was found expedient to let the alcohol on the surface of the ovary evaporate to a certain point in order to secure accurate measurements. After each corpus luteum was measured the ovary was returned momentarily to the alcohol to prevent desiccation. In order to avoid measuring any corpus luteum twice and omitting another, it was found advisable to make a sketch of the ovary and assign a number to each corpus luteum. Since the caliper used reads only to one decimal place, only the first two figures in our values are of real significance.

Under conditions in which it was impossible to distinguish the most recently formed corpora lutea from others, intraperitoneal injections of the vital dye, Dianil-blue 2R, were administered according to the method of Long and Evans ('22).

This report is based on measurements of corpora lutea in the ovaries of 266 rats under normal and experimental conditions. Normal rats include normal cyclic, pseudopregnant, normal pregnant, normal lactating and pregnant lactating animals. Experimental rats include those injected with Antuitrin-S, estrogens (theelin and stilboestrol) and prolactin.

OBSERVATIONS

Normal animals

1. Normal cyclic rats. Eight normal adult females all previously injected with Dianil-blue 2R, were sacrificed, two on each of the 4 successive days of the estrous cycle. The injections brought about diestrous periods ranging from 12

to 14 days. The animals were sacrificed after normal cycles had been resumed. Thus all unstained corpora lutea were those of ovulation. The measurements of these agree very closely, averaging 1.37 mm. in diameter. The maximum which they may attain is between 1.45 and 1.5 mm.

2. Pseudopregnant rats. Eleven mature females were rendered pseudopregnant by copulation with vasectomized males. Individuals were sacrificed on days 4, 5, 6, 7, 9, 10, 11 and 13 of the pseudopregnant period. The corpora lutea of these animals reach a diameter of 1.4 to 1.6 mm. by the fourth or fifth day of pseudopregnancy and maintain this size throughout the period. In none did the size of the corpora lutea exceed 1.66 mm.

3. Normal pregnancy. Fifty-four normal pregnant rats were sacrificed on different days during pregnancy and their corpora lutea measured. The curve based on these measurements (fig. 1) demonstrates that the corpora lutea of gestation remain constant at about 1.45 mm. until days 10 or 11. They then rapidly increase in size, reaching a diameter of about 2.0 mm. on the sixteenth day. This size is maintained until parturition.

4. Normal lactation. Thirty-three non-pregnant rats each suckling one to ten young, were sacrificed on days 7, 14, or 21 of the lactation period. The average size of their corpora lutea is 1.589 mm. (table 1). The number of young suckling does not affect the size of the corpora lutea in these rats since there are no significant variations between those suckling ten and those suckling one or two.

5. Prolonged gestation. The corpora lutea of ninety inseminated lactating rats suckling one to ten young were measured. Individuals were killed on days 7, 14, 21, 28 and 35 of the prolonged gestation period. In general, the larger the number of young suckling, the greater is the delay before corpora lutea begin to grow to the typical maximum size of pregnancy (tables 2 and 3). The measurements from these rats taken on the seventh and fourteenth days, correspond in general to those of non-pregnant lactating rats (table 1). Only

in cases where females were suckling one or two young, are the figures for the fourteenth day higher in the pregnant animals.

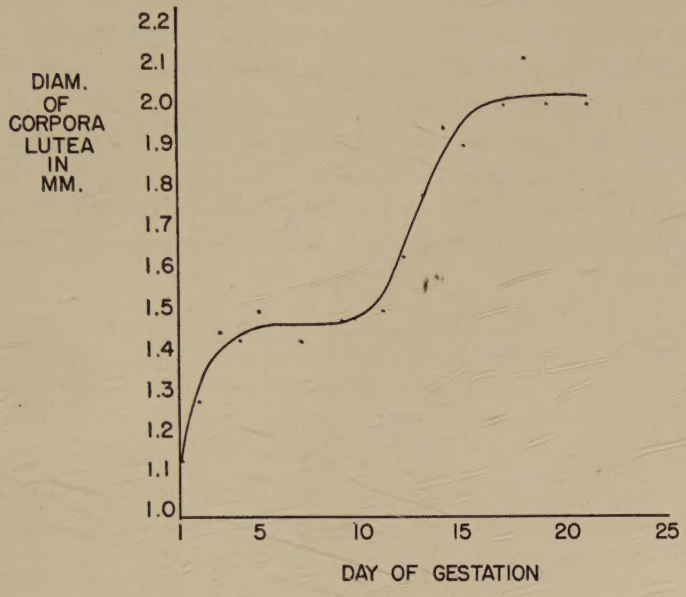

Fig. 1 Curve representing the growth of corpora lutea in the normal pregnant rat. (Based on measurements of corpora lutea in fifty-four pregnant animals.)

TABLE 1

Diameters of corpora lutea of normal lactating rats suckling from one to ten young. The figures in parentheses indicate the number of rats used to obtain each measurement.[1]

DAY KILLED	NUMBER OF YOUNG SUCKLING									
	1	2	3	4	5	6	7	8	9	10
			(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)
7	....	....	1.460	1.556	1.542	1.450	1.628	1.664	1.587	1.484
	(1)	(1)	(2)	(1)	(1)	(2)	(2)	(1)	(1)	(1)
14	1.667	1.590	1.581	1.660	1.621	1.685	1.624	1.635	1.675	1.621
			(1)	(1)	(1)	(1)	(4)	(2)	(1)	(1)
21	....	....	1.500	1.461	1.478	1.691	1.574	1.588	1.625	1.680

[1] Diameter expressed in millimeters.

The uteri of forty of these rats, all killed on the twenty-first day of gestation, were selected for further examination. All showed macroscopically visible implantation sites, indicating that the embryos had progressed beyond the blastocyst stage and had implanted. Embryos were removed from the uteri. By comparing them with normal embryos, it was possible to estimate the time of implantation within ½ day since during normal pregnancy implantation takes place on day 6 (Huber, '15). The diameter of the corpora lutea on the twenty-first day of pregnancy is definitely correlated with the day of implantation (table 3). Hence, the larger the embryo on a given day, the greater is the diameter of the corpora lutea.

TABLE 2

Diameters of corpora lutea of inseminated lactating rats suckling from one to ten young. The figures in parentheses indicate the number of rats used to obtain each measurement.[1]

DAY KILLED	NUMBER OF YOUNG SUCKLING										
	0	1	2	3	4	5	6	7	8	9	10
	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)
7	1.432	1.512	1.496	1.504	1.564	1.606	1.600	1.571	1.378	1.508	1.330
	(3)	(1)	(3)	(2)	(3)	(2)	(1)	(1)	(2)	(1)	(1)
14	1.950	1.803	1.722	1.678	1.519	1.595	1.591	1.628	1.605	1.650	1.583
	(7)	(4)	(3)	(4)	(5)	(5)	(5)	(5)	(6)	(7)	(7)
21	2.063	2.092	2.079	2.134	1.992	1.862	1.847	1.721	1.621	1.629	1.557
					(1)	(2)	(1)	(1)	(1)	(2)	(1)
28	....	....	....	....	2.103	2.097	1.993	2.198	2.031	1.971	2.015
								(1)	(1)	(1)	
35	....	....	....	....	....	....	....	2.149	1.958	2.122	....

[1] Diameter expressed in millimeters.

Injected animals

1. Antuitrin-S. The ovaries of thirty-eight rats, from another experiment, which were injected with Antuitrin-S, were examined. These rats included twenty-six injected during prolonged gestation, nine injected during normal pregnancy and three injected when undergoing normal estrous cycles. The number of injections ranged from four to twenty and the daily dose from 10 to 100 RU.

Although a series of injections of Antuitrin-S results in heavily luteinized ovaries, the appearance of these structures is atypical. The number of corpora lutea in all cases is excessive, ranging from twenty-five to sixty per ovary. There is a striking lack of uniformity in the size of the corpora lutea in any one ovary.

TABLE 3

Diameters of corpora lutea of rats undergoing prolonged gestation and sacrificed on the twenty-first day. The correlation between size of corpora lutea, day of implantation and number of suckling young is shown.

RAT NO.	NO. YOUNG SUCKLING	DAY OF IMPLANTATION	DIAMETER OF CORPORA LUTEA [1]	RAT NO.	NO. YOUNG SUCKLING	DAY OF IMPLANTATION	DIAMETER OF CORPORA LUTEA [1]
D305	10	20	1.442	D484	4	14½	1.791
D610	10	19½	1.597	D521	6	13	1.986
D453	10	19½	1.540	D468	7	12	1.894
D185	8	19	1.667	D192	7	12	1.926
D363	8	19	1.650	D572	5	12	2.180
D217	5	19	1.675	D458	6	11	2.175
D233	5	19	1.652	D506	3	11	2.020
D472	9	18	1.535	D530	4	10	2.233
D287	8	18	1.591	D212	3	10	2.178
D584	9	17½	1.830	D204	3	10	2.101
D477	7	17	1.587	D400	5	9½	2.163
D197	5	17	1.557	D205	4	9	1.977
D270	8	17	1.668	D412	3	9	2.232
D462	8	15½	1.657	D199	2	7	2.055
D230	6	15½	1.675	D652	2	7	2.031
D225	7	15½	1.623	D429	2	6	2.068
D219	6	15	1.617	D522	1	6	2.215
D166	4	15	1.853	D457	1	6	2.095
D352	5	14½	1.759	D220	1	6	2.009
D247	6	14½	1.781	D299	1	6	2.050

[1] Diameter expressed in millimeters.

In the injected rats undergoing prolonged gestation, corpora lutea measuring 2.0 mm. or more were found only in cases where fetuses were larger than those of a normal rat on the sixteenth day of pregnancy. The same condition obtained in the injected normal pregnant animals. The ovaries of all these injected rats contained many small corpora lutea in

addition to the large ones. In those animals in which the embryos had resorbed or were being resorbed, no corpora lutea attained the maximum diameter.

The three rats undergoing normal estrous cycles were injected daily with 20 RU Antuitrin-S beginning on the day of estrus. All were sacrificed on the twenty-first day. Two were injected for 8 days and one for 20 days. Their corpora lutea ranged from 1.075 mm. to 1.600 mm. and 1.770 mm. to 2.050

TABLE 4

Diameters of corpora lutea of normal lactating rats injected with estrogens beginning on the day of parturition.

RAT NO.	HORMONE INJECTED	NO. OF DAYS INJECTED	DAY KILLED AFTER HEAT	DAILY DOSE	AVERAGE DIAMETER CORPORA LUTEA
				mg.	*mm.*
D752	.....	..	15	....	1.734
D815	.....	..	15	....	1.625
D780	Theelin	14	15	.025	1.864
D756	Theelin	14	15	.050	1.991
D982	Theelin	11	12	.100	2.033
D810	Theelin	14	15	.100	2.252
D785	Theelin	14	15	.200	2.229
D773	Theelin	14	15	.200	2.353
E 13	Theelin	13	14	.500	2.131
D747	Stilboestrol	14	15	.025	2.036
D749	Stilboestrol	14	15	.050	2.122
E 26	Stilboestrol	5	6	.100	1.739
E 6	Stilboestrol	9	10	.100	1.771
D998	Stilboestrol	11	12	.100	1.956
D731	Stilboestrol	14	15	.100	2.122
No. 6	Stilboestrol	14	15	.200	2.097

mm. respectively. The latter was the only rat injected with Antuitrin-S that had corpora lutea measuring 2.0 mm. in diameter without having well developed fetuses in the uterus.

Since the above observations showed that administration of Antuitrin-S brings about an atypical condition of the ovaries which in no case simulates that of the latter part of pregnancy, injections of estrogens, which are also known to bring about growth of corpora lutea, were undertaken.

2. Estrogens. Fourteen non-pregnant rats, each suckling six young, were injected daily with theelin or stilboestrol beginning on the day of parturition. They were sacrificed on days 6 to 15 and their corpora lutea measured (table 4). Two normal lactating rats, each suckling six young, served as controls.

Two rats made pseudopregnant by copulation with vasectomized males, were injected with .02 mg. and .04 mg.

TABLE 5

Diameters of corpora lutea of normal lactating rats injected with prolactin beginning on the day of parturition.

RAT NO.	NO. OF YOUNG SUCKLING	AMOUNT INJECTED DAILY	NO. OF INJECTIONS	DAY KILLED	AVERAGE DIAMETER CORPORA LUTEA
		R.U.			*mm.*
D718	1	5	20	21	1.782
D744	2	10	20	21	1.787
D707	2	10	20	21	1.687
E 57	1	10	15	16	1.702
D714	1	10	20	21	1.671
E 48	3	10	20	21	1.744
E111	4	10	20	21	1.834
E 72	2	20	20	21	1.733
D769	1	20	20	21	1.625
D811	2	20	20	21	1.785
D702	3	20	20	21	1.722
D778	3	20	20	21	1.641
D708	4	20	20	21	1.745
D706	1	20	20	21	1.648

stilboestrol respectively from the seventh to the twentieth days of pseudopregnancy. No vaginal smears of the estrous type were observed during this interval. The rats were sacrificed on the twenty-first day and the corpora lutea measured. Those of the former averaged 1.915 mm. and those of the latter, 2.228 mm.

The data from these estrogen injected non-pregnant rats show that the size of the corpora lutea is directly correlated with the amount of hormone administered and the length of time during which injections are given. These corpora lutea

are of a size comparable to those present during the latter half of pregnancy; a size not observed in non-pregnant animals under normal conditions. Moreover, the number of corpora lutea in these ovaries is similar to the number found during normal pregnancy.

3. Prolactin. Fourteen non-pregnant lactating rats suckling small litters were injected daily with 5 to 20 Riddle Units of prolactin beginning on the day of parturition. All but one of these animals were sacrificed on the twenty-first day. The corpora lutea are slightly larger than those of normal lactation, averaging 1.721 mm. in diameter. The various doses administered had no differential effects (table 5).

DISCUSSION

Our observations on the growth of corpora lutea of the rat during normal pregnancy confirm those of Long and Evans ('22) and Swezy ('33). It is noteworthy that the growth curve of the corpora lutea of the pregnant rat (fig. 1), differs markedly from that of the rabbit (Pincus and Berkman, '37) in which there is a continuous rise up to the twenty-eighth day of pregnancy and not an abrupt increase around the half-way mark.

The retarded growth of the corpora lutea of rats during prolonged gestation is of interest. Kirkham ('16 a, b, '18) and Enzmann, Saphir and Pincus ('32) have shown that implantation of fertilized ova is delayed in pregnant lactating rats and that the delay is correlated with the number of suckling young. The latter investigators report that after implantation finally occurs, the growth curve of the developing young is indistinguishable from that of normal pregnancy.

The interval between fertilization and the time when the corpora lutea begin to increase in size is prolonged in the lactating rat; but after implantation has occurred the growth curve of the corpora lutea follows that of normal pregnancy. For example, the uterus of rat D192, suckling seven young, contained embryos, on the twenty-first day of gestation, at a state of development comparable to the fifteenth day of normal

pregnancy. The corpora lutea of this rat averaged 1.926 mm., which corresponds closely to the size of corpora lutea on the fifteenth day of a non-lactating pregnancy (1.907 mm.). Corresponding ovaries in the two groups during the latter part of pregnancy can in no way be distinguished as to either size or number of corpora lutea.

It is known that pituitary and placental hormones bring about development of corpora lutea. In a recent publication, Astwood ('41) reports a pituitary fraction called luteotrophin which brings corpora lutea to a functional state. Evans, Simpson and Lyons ('41) identify this with the lactogenic hormone. Injections of prolactin in the form used in our experiments, however, do not give the ovarian picture seen during the latter half of pregnancy, for in no case were large corpora lutea formed.

That the placenta may be the source of the factor causing luteal growth is suggested by the fact that during prolonged gestation when the formation of the placenta is delayed, the increase in size of the corpora lutea is also delayed. Astwood and Greep ('38, '39) have shown that the placenta contains a gonadotrophic substance (cyonin) which is independent of the pituitary gland. They have suggested that this substance enlarges and maintains the corpora lutea during the latter half of pregnancy in the rat. Little is known concerning the nature of this substance except that it is not estrogen since it does not cause vaginal estrus in spayed females (Greep, '41).

The atypical ovaries found after injection of Antuitrin-S indicate that this substance of placental origin is probably not responsible for the development of the large corpora lutea of the latter half of pregnancy. Moreover, Selye, Collip and Thomson ('34) have reported that only during the latter half of pregnancy do the excessively large numbers of corpora lutea formed in such ovaries grow to a large size.

The luteinizing effects of estrogens have been observed by several investigators but only Wolfe ('35) has published accurate measurements. He injected Progynon-B into mature females and obtained a normal number of large corpora lutea

of the same size as those of the latter half of pregnancy. Selye, Collip and Thomson ('35) likewise observed that estrogen injections increased the size but not the number of corpora lutea in normal lactating rats.

Merkel and Nelson ('40) report that estrogen injected into normal mature female rats produces changes similar to the luteal effects found in pseudopregnancy. This condition, described as one in which the ovaries contain a number of "large" (?) corpora lutea and in which the estrous cycles are suppressed, lasts about 20 days. No new corpora lutea are formed by this treatment. The estrogen is thought to act by releasing luteinizing hormone from the pituitary gland since similar results were not obtained in hypophysectomized animals.

That corpora lutea formed under the influence of estrogen are probably functional, is indicated by the work of Robson ('37) who found in rabbits hypophysectomized during pseudopregnancy, that luteal function as expressed by the placentomata response, can be maintained by injections of estrogen. Greep ('41) reported that estrogen injections in the rabbit prevent the luteal involution which occurs after hysterectomy during the second half of pregnancy even if the animal has been hypophysectomized. It seems that the action of estrogen on corpora lutea, at least in the rabbit, is direct and not mediated through the hypophysis.

No experimental work of which the authors are aware has conclusively shown what factors are essential for the growth of corpora lutea during pregnancy. Since estrogens injected into normal females give the only response comparable to that seen during the latter part of normal or delayed pregnancy it may be presumed that they are involved in the normal stimulation of luteal growth.

SUMMARY

1. Corpora lutea of ovulation average 1.37 mm. in diameter.

2. Corpora lutea of pseudopregnancy average 1.517 mm. in diameter.

3. Corpora lutea of normal pregnancy remain constant at about 1.45 mm. until days 10 or 11 when they undergo a rapid increase in size and remain constant at about 2.0 mm. from the sixteenth day until parturition.

4. Corpora lutea of normal lactation average 1.589 mm. The size is not influenced by the number of suckling young.

5. The increase in size of corpora lutea in prolonged gestation is comparable to the condition in normal pregnancy except that because of later implantation, the gestation period is lengthened and the increase in size of the corpora lutea is correspondingly delayed. This delay is in general correlated with the number of suckling young.

6. Injections of Antuitrin-S, estrogens and prolactin, show that only with estrogens is the typical ovarian picture of the latter half of pregnancy obtained in the non-pregnant rat.

7. The size of the large corpora lutea produced by the estrogen treatment is directly correlated with the dose and the length of time during which it is administered.

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REPORT OF A CASE IN WHICH ALL PULMONARY VEINS FROM BOTH LUNGS DRAIN INTO THE SUPERIOR VENA CAVA

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ONE FIGURE

To the growing list of anomalous pulmonary veins the following case observed in a 7-day infant, may be added [1].

Post mortem examination revealed but one major abnormality. The four normal pulmonary veins converge as if to enter the left atrium. Instead they unite behind the heart to form a common pulmonary trunk 3 cm. long which arches over the dorsal surface of the right bronchus and empties into a normal vena cava superior about 1 cm. above its end (fig. 1). The common pulmonary trunk receives an additional tributary from the superior lobe of the right lung.

The lungs are normal in all respects. The surface vessels are rather conspicuous. The arrangement of bronchi, arteries, and veins at the root of each lung is normal save for the additional vein from the right superior lobe already referred to. The greater part of the anomalous common pulmonary trunk lies in a well-marked azygos groove of the right lung, and

[1] The infant was born October 7th. Delivery was spontaneous and respiration also. Though the baby cried and breathed spontaneously, there was a slight dusky cyanosis present from birth. Physical examination revealed no other abnormalities than a heart rate of 180. The infant's progress was fairly good. He nursed at the breast and took complementary feedings quite well. He became slightly jaundiced October 11th. At times when crying he would become definitely cyanosed, at other times he would cry vigorously without very much change. On October 14th, his abdomen appeared very much distended. His cry became weak and shrill and the cyanosis severe. He died on the same day at the age of 7 days.

therefore has the position of the usual terminal stem of the right bronchial and azygos veins. Whether an azygos vein joined the common pulmonary trunk could not be determined.

The heart is normal save for minor changes resulting from the unusual venous return. The right atrium is much enlarged, but otherwise normal. It receives the usual veins. The left atrium is small and shrunken. It receives no veins of any kind. It is however in free communication with the right atrium through a fully patent foramen ovale. There is nothing peculiar about the fossa ovalis. Its membranous floor flaps loosely and would permit free passage of blood from the right into the left atrium. There is no other abnormal defect in the interatrial septum at any place. Both atrioventricular canals and their valves are normal.

The two ventricles, while normal in structure, are abnormal in size. The right ventricle overshadows the left about as much as the left normally does the right. The walls of the right ventricle have about twice the thickness of the left. The interventricular septum is normal in every respect.

The pulmonary artery is nearly twice the diameter of the aorta at their origins, and the conus arteriosus is roomier in proportion. The branches of both vessels are normal. The ductus arteriosus shows a distinct constriction which would not admit a probe; apparently there had been a physiological if not anatomical closure of the ductus sometime before death.

In brief there is one really significant abnormality. The four otherwise normal pulmonary veins are diverted over a new common trunk into the superior vena cava.

Such a total diversion of the pulmonary venous return into the vena cava superior, without other grave abnormalities of the heart and great vessels, seems to be rare. The case 2 of Brody ('42) comes nearest to it. In Brody's case, the four veins drain through a short trunk 6 mm. long into the vena cava superior immediately above the entrance of the vein into the right atrium. The short trunk passes ventral to the right bronchus, and therefore does not follow the course of the right

bronchial and azygos vein as in our case. The difference is slight but significant.

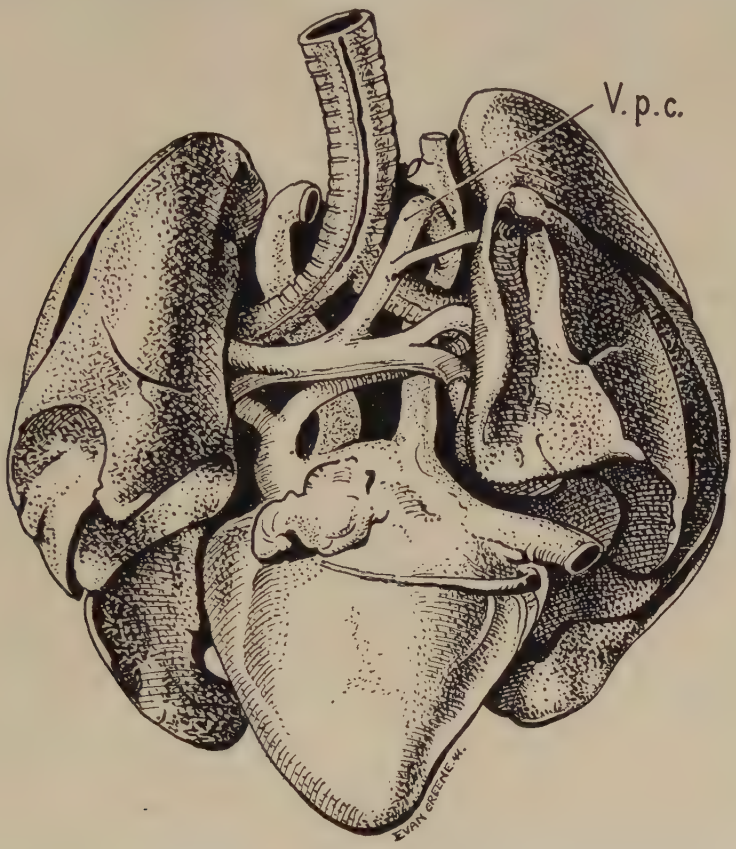

Fig. 1 Heart and lungs of a 7-day-old child, with the four pulmonary veins draining through a common pulmonary vein (V.p.c.) into the superior vena cava. The lungs are lifted up to display the vessels of the heart.

The case of Goltman and Stern ('39), which is one of partial situs inversus and numerous cardiac abnormalities, includes a left common pulmonary trunk running dorsal to the left bronchus into a persisting left superior vena cava. In the case of Barge and Oijen ('32) the left pulmonary veins cross to the right lung and both lungs are drained into the superior vena cava by a "right pulmonary" vein. The vein passes over the right bronchus as in our case. On the other

hand, in the ectopic heart described by Wilson in 1798, the pulmonary veins form a common trunk which passes ventral to the bronchi and ends in an irregular vena cava superior, somewhat as in Brody's case. Other possible cases are those of Ono ('22) and Epstein (1886).

DISCUSSION

A comparison of our case with that of Brody, and the others just referred to, emphasizes the fact that abnormalities of pulmonary veins involve not only the heart and pulmonary veins, but the bronchial veins as well.

The development of the true pulmonary veins has been studied by Flint ('06), Brown ('13), Squier ('16), Buell ('22), Chun Chang ('31) and Davies and MacConaill ('37). There is no correspondingly detailed study of the bronchial veins. We are still dependent upon the fundamental work of Brown ('13), which is confined to the first stages. According to Brown, the tracheal-pulmonary outgrowth is covered by the same vascular plexus as the rest of the primitive foregut. The pulmonary vascular plexus is drained not only by a primitive pulmonary vein into the heart, but also by venous taps into the anterior and posterior cardinal veins, and into the inferior vena cava. The primitive pulmonary vein is later tranferred to the left atrium and then absorbed into it. The four branches of the primitive pulmonary vein persist as adult vessels. One of the taps into the posterior cardinal vein of each side becomes the later bronchial vein. The bronchial veins therefore open into the adult azygos or hemiazygos vein, which are derived, in part at least, from the cardinal vessels.

The anomalous pulmonary vein in our case is probably an hypertrophied right bronchial vein. The true pulmonary vein was normally formed in the earliest stages. But before the absorption of the parent trunk by the left atrium could draw the pulmonary branches into it, the right bronchial vein had enlarged, captured the four vessels, and diverted their blood into the right posterior cardinal vein. When the primitive pulmonary trunk withered away, the arrangement shown in figure 1 would be produced.

On the other hand, Brody's case 2, which at first sight is so much like our own, is probably a true common pulmonary trunk which has shifted to the right on to the vena cava superior following some mishap in the formation of the atrial septum and the remodelling of the sinus venosus and right atrium. This is Brody's interpretation, with which we agree. Such a common pulmonary trunk lies ventral to the right bronchus. It does not take the circuitous course over the bronchus found in our case, and indeed could not.

The implication of bronchial veins in abnormalities of pulmonary drainage was recognized long ago by Johnston ('15) and by others before him. The contribution of the bronchial veins to such anomalies may be more frequent than generally realized. Brody has tabulated 106 cases of abnormal pulmonary drainage. A study of the table brings out the fact that less than a third of such veins enter the right atrium or coronary sinus. Except for a few into the portal vein and inferior vena cava, the rest are about equally divided between the vena cava superior, right innominate and azygos veins on the one hand, and the left innominate, left subclavian and persisting left superior vena cava on the other. For the majority of anomalous pulmonary veins, the bronchial vein or some other persisting channel of the early cardinal system may be suspected as the significant factor.

SUMMARY

A rare case of anomalous pulmonary vein is described, in which the four pulmonary veins drain by a single trunk dorsal to the right bronchus into the superior vena cava. The trunk is probably an hypertrophied right bronchial vein. Such abnormal bronchial veins are more significant than generally supposed.

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DELAYED IMPLANTATION IN THE LONG-TAILED WEASEL (MUSTELA FRENATA), THE SHORT-TAILED WEASEL (MUSTELA CICOGNANI), AND THE MARTEN (MARTES AMERICANA) [1]

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TWO PLATES (TWELVE FIGURES)

INTRODUCTION

In a review paper Hamlett ('35) states that in mammals an unusually long period between the time of mating and the time of birth of the young, if the young are not extremely large at birth, is to be explained by either of two phenomena, delayed fertilization or delayed implantation. Among the American species known to show a delayed implantation the badger and the armadillo have been most fully studied (Patterson, '13; Hamlett, '32 a and b). In both of these animals the embryos remain in the free blastocyst stage for several months before implantation.

Hamlett ('35) also states that the marten (Martes americana) probably has this type of development as it has been shown that breeding takes place during July, August, or September and that the young are not born until the following April or May (Ashbrook and Hanson, '27 a and b, '30; and Enders and Leekley, '41). Hamlett ('35) observed corpora lutea in martens which died in late summer or fall after breeding. Although he was unable to find free vesicles in the uteri of these animals, he felt that the presence of the corpora lutea made it seem probable that the case was one of delayed im-

[1] This study has been aided by a grant from the Wisconsin Alumni Research Foundation administered by R. K. Meyer, and by grants from the Elizabeth Thompson Science fund and the National Research Council Committee for Research in Problems of Sex.

plantation rather than delayed fertilization. He states, however, that the recovery of unimplanted blastocysts at a considerable time after the breeding period is necessary before we can be sure of the type of delayed development.

Warren ('32) makes the suggestion that perhaps the long-tailed weasel (Mustela frenata) may breed in the summer time. Hamilton ('33) is of the same opinion, since he had a captive female which was not allowed access to a male during late winter and spring. This animal produced young and the shortest possible gestation period was 70 days. Hamlett ('35) observed blastocysts in two female long-tailed weasels killed in July. He was not sure whether these were to lie dormant for a time and develop during the next spring, or whether they were active embryos representing a litter to be born later on in the same season. According to Hamilton ('33) the short-tailed weasel (Mustela cicognani) seems to have the same type of sexual cycle as the long-tailed.

It is the purpose of this paper to show that the two above mentioned weasels and the marten possess a discontinuous type of embryological development.

MATERIALS

During the past 4 years I have been observing weasels of the two above mentioned species with respect to the molt and the sexual cycle. Thirty-six captive weasels have been used during this time, some individuals having been kept longer than 2 years. Most of the animals have been from the long-tailed species and only males of the short-tailed species have been available. In addition some fifty carcasses including both sexes of both species taken by trappers have been studied. Most of these carcasses have been preserved by the trapper in 10% formalin after opening the abdominal wall. Since many of the carcasses were to remain in the preservative for several weeks and it has been desirable to preserve skeletal material as well as the reproductive tracts, preservatives containing acids have been avoided. Most of the material preserved in this way has been satisfactory for gross histological

work. The majority of these animals were taken in western Montana.

Reproductive tracts from twelve martens captured in the winter have also been studied. Nine of these were preserved by the trappers and three were kindly given me by Dr. J. S. McFarland. According to Dr. E. Raymond Hall and Walter W. Dalquest who examined skulls from these martens, two species are represented in the series. The specimens from Flathead county, Montana are referable to Martes americana, whereas those from Ravalli county, Montana are referable to Martes caurina. Hall ('36) believes that careful taxonomic study may eventually show that these two represent subspecies of the same species.

I gratefully acknowledge helpful suggestions rendered by Dr. R. K. Meyer, Dr. H. W. Mossman, and Dr. L. G. Browman. I am especially indebted to Margaret H. Wright who performed most of the histological technique.

RESULTS

Mustela frenata. Histological study of the ovaries of several females taken during the months of September, October, November, December, and January shows corpora lutea in all (fig. 8). Sections of the uteri show the presence of unimplanted blastocysts in all of the specimens which are sufficiently well preserved (six out of eight).

Of thirteen live females which have been sent to me by trappers during the fall or winter, all have ultimately proved to be pregnant without further contact with a male. One captive female which was caged with an adult male during the summer and isolated on September 1 produced a litter of young the following May 1. The shortest possible gestation period in this case was 252 days. During the following summer three captive pairs were observed to copulate in July or August, but the gestation periods of these animals will not be known until spring. Hamilton ('33) states that the young of the long-tailed weasel in New York state are born

from mid-April to the second week in May. Three normal control animals used in this study and kept at Missoula, Montana had their young on April 17, May 1, and May 3. This indicates that the young are born at about the same time in these two regions. There is no evidence that this species may produce more than one litter per year.

Since all of the twenty-two females, both carcasses and live animals, sent in by trappers during the fall and winter months were pregnant, one suspects that young animals become pregnant during their first summer. Two sources of evidence prove this to be true. Young female weasels can be distinguished from older animals for 8 to 10 months by differences in the maturity of the skull (fig. 1). Three animals taken during fall or winter with skulls of the immature type were pregnant. Also two females born in captivity in May showed in August the enlarged vulvas of animals in heat. One of these animals mated when caged with an adult male. The other, no. 75, which was also allowed access to the same male in August, died October 27 and blastocysts were found in the uterus (figs. 1, 4, 5, and 6).

A more detailed description of the sexual cycle of this species will be published as soon as a few more stages are obtained.

Mustela cicognani. Six carcasses of females of this species taken during October, December, January, and February have been studied. The ovaries of five of the six contain corpora lutea (figs. 7 and 10) and blastocysts are visible in the uteri of four of the five (fig. 3). The animal whose ovaries do not show corpora was taken in October and there seems to be no possibility that she could have become impregnated later on during the fall, since adult males of this species are not sexually active after September 1 (unpublished data of the writer). The skull of this non-pregnant animal indicates that she was a young one of the year, but since I can not determine the ages of the other specimens it is not possible at this time to say at what age females reach sexual maturity. However, it is apparent that this species possesses the same delayed

implantation type of development characteristic of the larger species, Mustela frenata.

Martes americana and Martes caurina. Seven reproductive tracts of female martens have been studied, five of M. americana and two of M. caurina. Four of the seven tracts contain corpora lutea in the ovaries and blastocysts in the uteri (figs. 2 and 9). None of these specimens are accurately dated although two of the four were taken between December 27, 1940 and January 31, 1941. The four pregnant martens were all of the species M. americana so it is not possible to say that M. caurina possesses the same type of delayed development. However, it is believed that the three non-pregnant martens, one of M. americana and two of M. caurina, were young animals and were not pregnant because they had not reached sexual maturity. Enders and Leekley ('41) mention captive females of M. americana at the U. S. Bureau of Animal Industry fur farm at Saratoga Springs, New York that seemed not to come into heat at 1 or even 2 years of age.

The testes of adult male martens taken at the same time as the above females are in a non-breeding condition. Therefore it is assumed that the blastocysts observed had been present since the close of the breeding season in September (Enders and Leekley, '41) and that the delay in the birth of young martens is due to a delayed implantation.

DESCRIPTION OF THE BLASTOCYSTS

Mustela frenata. Most of the blastocysts in all three species show varying degrees of disintegration due to the method of preserving the carcasses. However, two specimens of Mustela frenata show well preserved embryos. The vesicles resemble those described by Hamlett ('32 b) from the badger (figs. 4 and 6). The zona pellucida is very conspicuous and averages 4.7 microns in thickness in eight blastocysts (fig. 6). The blastocysts are at least partly collapsed in all of the specimens. The average diameter of eight embryos is 378 microns. The diameter of the embryos is apparently larger than this, however, as evidenced by the enlarged areas in the lumina of

several uteri (fig. 5). It is assumed that the embryos normally completely fill these spaces. If so they would be about 600 microns, and smaller in diameter than those described by Hamlett ('32 b) for the badger (950 to 975 microns).

The cells of the trophoblast are very much flattened and barely visible (fig. 6) except for the nuclei which are easily seen. The total number of nuclei in the trophoblast varies from 125 to 174 and averages 151 in six blastocysts. The inner cell mass is very small in comparison with the size of the whole embryo. In six specimens it averages 71 microns in diameter and 16 microns in thickness. In these same specimens the number of nuclei in the mass ranges from 18 to 28 and averages 24.

Mustela cicognani. The preservation of the specimens of this species does not permit a detailed description as all are at least partially degenerated (fig. 3). Corresponding with smaller body size the embryos are all somewhat smaller than those of M. frenata. The average diameter of four collapsed specimens is 318 microns. The zona pellucida averages 4.5 microns in the same specimens. Only one embryo is well enough preserved to allow a count of the number of cells, there being 147 cells although those of the inner cell mass can not be distinguished from those of the trophoblast.

Martes americana. The blastocysts of this species are larger than those of the weasels. The embryos all show some disintegration. The average diameter of four specimens is 409 microns but like those of the weasels they were undoubtedly larger before they became collapsed (fig. 2). Also there are more cells present, the number ranging from 291 to 506 is four specimens. Those of the inner cell mass can not be distinguished from those of the trophoblast. The zona pellucida in the same specimens averages 6.9 microns thick.

CORPORA LUTEA

The corpora lutea of female weasels of both species and of martens with unimplanted blastocysts are small and inconspicuous (figs. 7, 8, and 9). In no case do they appear to pro-

trude from the wall of the ovary. The corpora of M. frenata and M. cicognani show small inactive appearing cells under high magnification (figs. 10 and 11).

The corpora of the marten differ considerably from those of the weasels. The cells are highly vacuolated (fig. 12) resembling those described from the corpora in the cat after the fiftieth day of pregnancy (Dawson, '41). According to Dawson the fatty infiltration seen in the corpora of the cat seems to be evidence of functional inactivity. It seems likely that the corpora in the marten undergo some regenerative change or are replaced by new or accessory corpora before implantation takes place. No ovaries of any of these species have been studied after implantation has occurred.

DISCUSSION

Deansley ('35) in a study of the sexual cycle of the stoat (Mustela erminea), concluded that females mate during the summer, fall, or early spring. However, she believed that only matings occurring in early spring are fertile, all others being sterile. Corpora lutea were seen in the ovaries of both young and old females after these so-called sterile matings. These corpora were smaller and the cells were smaller than those of the animals in advanced pregnancy. She does not suggest that there might be a delayed implantation in this animal.

Watzka ('40) also studied the sexual cycle of the stoat. He found that some female stoats become pregnant during June or July and after a delay of about 7 months, implantation occurs and the young are born in April or May. Other females become pregnant not during the summer but during March after which they have their young following a gestation period of 8 to 9 weeks.

Since Watzka's conclusions were based partly on observation of live animals and Deansley's entirely on autopsy data, it is suspected that Watzka's interpretation is more accurate. There seems to be no reason why the morphological data presented by Deansley can not be interpreted in the same way, especially since she made no reference to delayed implantation

as a possible explanation of the summer matings which were not followed immediately by an active pregnancy.

It is of interest to speculate whether the short gestation period described by Watzka might occur in the two American species of weasels, M. frenata and M. cicognani. As previously mentioned, all wild specimens of M. frenata obtained in the fall and winter have been pregnant. Certainly, then, spring breeding must be rare in this species if it occurs at all. However, two captive females which were not bred during the summer showed in the spring the enlarged vulva of animals in heat. These animals were not mated at this time. Since sperm do not begin to appear in the epididymides of males of this species until late March and are not abundant until mid-April (author's unpublished data) the young resulting from a spring mating would not be born until late May at the earliest if an 8 or 9 weeks gestation period occurred. I know of no evidence for the birth of young after early May in this species. The available data on M. cicognani do not permit me to say whether a spring breeding period might occur.

SUMMARY

1. The long-tailed weasel breeds during July or August and the young are born the following April or May. During the larger part of this long period the embryos lie dormant in the uterus as unimplanted blastocysts. The young female weasel mates when 3 or 4 months old, so that all females of this species may produce young in the spring.

2. There is also a delay before the implantation of the embryos of the short-tailed weasel since blastocysts are to be found in the uteri of female animals taken during the fall and winter months when males are not sexually active.

3. The presence of blastocysts in the uteri of female martens (Martes americana) taken during the winter supplements the breeding data of other workers to prove that there is a delay in implantation in this species. Two females of Martes caurina were not pregnant, but it is thought that these animals were sexually immature.

4. The corpora lutea of the two weasels and of M. americana are small and inconspicuous. The cells of the corpora in the weasels are not vacuolated, but those of all four pregnant martens are highly vacuolated.

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PLATE 1

EXPLANATION OF FIGURES

1 Skulls from females of Mustela frenata. The skulls at the left are from young animals, those at the right from animals older than 1 year. Notice the more prominent saggital crests on the skulls of the older animals, more constriction in the post orbital region, and the less rounded crania. Animal 57 was taken in January and is assumed to be about 9 months old. Blastocysts were found in the uterus of this animal. Animal 75 was born in captivity on May 1, 1941, an adult male was placed in her cage on August 20 and was removed 2 days later. She died October 27 and blastocysts were found in the uterus. Animal 33 died after more than a year in captivity in October, 1941 and was thus known to be at least 18 months old. This weasel was pregnant during the spring previous to her death. Animal 61 was taken in January and was assumed to be at least 21 months old. Blastocysts were found in the uterus of this animal. Approximately three-fifths natural size.

2 Blastocyst of Martes americana. This blastocyst is partly collapsed and the preservation is not good. The animal was taken between December 27, 1940 and January 31, 1941. × 60.

3 Blastocyst of Mustela cicognani. This blastocyst is completely collapsed and the preservation is poor. This animal was taken in January, 1941. × 60.

4 Blastocysts of Mustela frenata. These blastocysts are only partly collapsed and are the best preserved specimens available. This animal was taken in October, 1941. × 60.

5 Uterus of Mustela frenata. Notice the inflated areas in the lumen where the blastocysts are located. It is thought that the blastocysts filled these spaces in the living animal. There are three blastocysts visible. × 25.

6 Blastocyst of Mustela frenata. The inner cell mass and the thick zona pellucida are conspicuous. A portion of the very thin trophoblast with a single nucleus is seen on the left. × 250.

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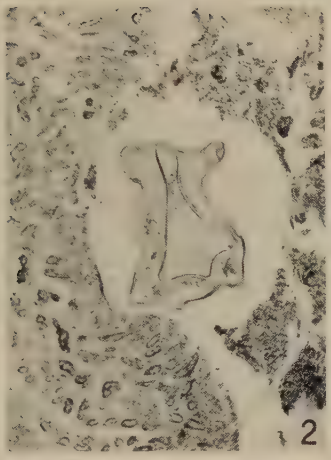
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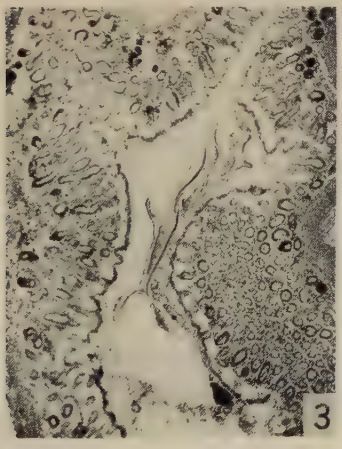
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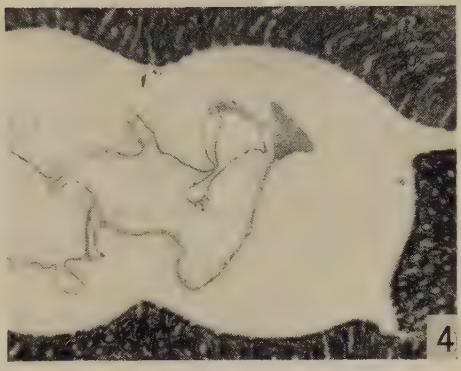
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PLATE 2

EXPLANATION OF FIGURES

7 Ovary of Mustela cicognani to show a corpus luteum of an animal with unimplanted blastocysts. Animal killed January, 1941. × 50.

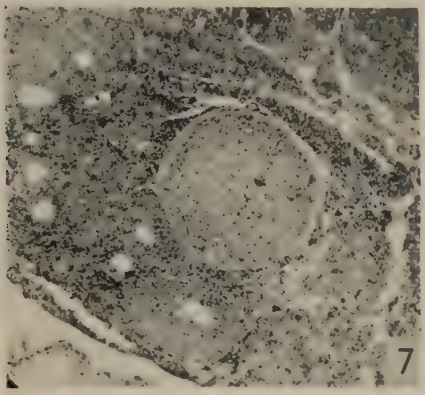
8 Ovary of Mustela frenata to show a corpus luteum of an animal with unimplanted blastocysts. Animal killed in January, 1941. × 50.

9 Ovary of Martes americana to show a corpus luteum of an animal with unimplanted blastocysts. Animal killed between December 27, 1940 and January 31, 1941. × 50.

10 Same section as figure 7 under higher magnification. Notice the small luteal cells and the lack of vacuolation. × 250.

11 Same section as figure 8 under higher magnification. Notice the small luteal cells and the lack of vacuolation. × 250.

12 Same section as figure 9 under higher magnification. Notice the high degree of vacuolation. × 250.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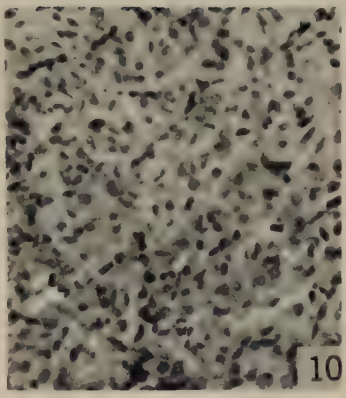
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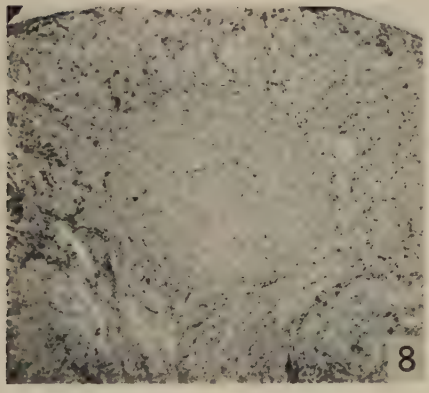
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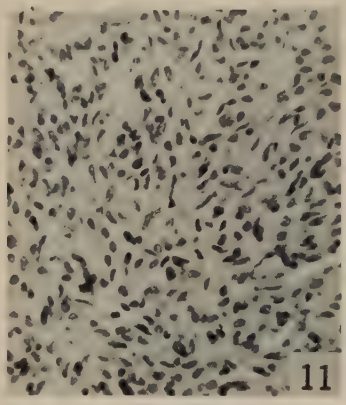
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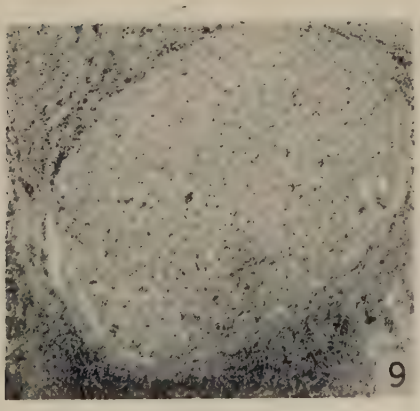
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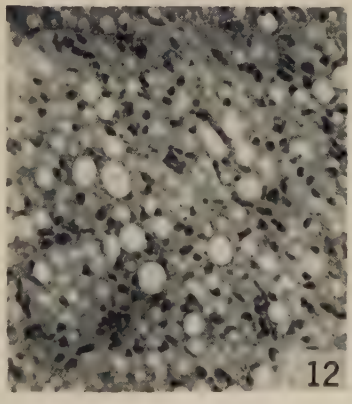
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CHANGES IN THE FIBRILLAR TISSUE OF THE ANTERIOR PITUITARY OF THE RAT ASSOCIATED WITH ADVANCING AGE [1]

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TWO PLATES (TWELVE FIGURES)

Recently, considerable interest has been shown in the effects of advancing age on the fibrillar connective tissue of various organs. Among the organs which have been studied in either the rat, mouse, or guinea pig are the uterus, cervix and vagina (Loeb et al., '38 and '39; Wolfe et al., '40 and '42; Burack et al., '41), the thyroid (Loeb and Simpson, '38, and Andrew, '40), the mammary gland (Loeb and Simpson, '38) and the thymus (Smith and Ireland, '41). In all instances there was an increase of fibrous tissue as the animal became older, the degree and rate of increase being variable in the different organs. Where silver impregnation methods were used, it was also found that a transformation of reticulum into collagen occurred; the degree and rate of this transformation was different in different organs. The present communication is concerned with the effect of advancing age on the fibrillar connective tissue of the anterior pituitary of the female rat.

Using the Bielchowsky-Maresch silver impregnation technic Corner ('20) found that the fibrillar material of the anterior pituitary of the cow consisted entirely of fine reticular fibrils

[1] These studies were aided by grants from the Josiah Macy, Jr. Foundation and the International Cancer Research Foundation.

intimately related to the endothelial cells lining the vascular channels. Fibroblasts were not found and he concluded that reticular fibrils were formed by, and lay in the cytoplasm of, the endothelial cells; similar findings were later reported by Rhinehart ('30). In the guinea pig Kirkman ('37) found a delicate reticular meshwork and a slightly denser collagenous network supporting the cords of anterior lobe cells. He did not definitely associate the fibrillar material with the vascular system other than to state that connective tissue fibers were always found between the anterior lobe cells and the capillaries.

The capillaries of the anterior pituitary are generally considered to be sinusoidal in nature and their lining cells phagocytic. Therefore, these cells are classed by most observers as components of the so-called reticulo-endothelial system although Sincke ('28) reported that they have no greater affinity for trypan blue than the endothelium found in the capillaries of striated or cardiac muscle.

MATERIALS AND METHODS

The pituitaries of seventy-six virgin female rats ranging in age from 35 to 884 days (the age groups were 35, 90, 180, 360, and 540 days and above) were fixed in 10% neutral formalin, embedded, sectioned serially at 6 microns and stained by Gömöri's ('37) silver impregnation technic. This method stains reticulum black and collagen rose red; fibers transitional between reticulum and collagen are purplish or blackish red.

OBSERVATIONS

In the anterior pituitary of the rat the fibrillar material was almost entirely reticular and was generally found only in association with the sinusoidal capillaries where the reticular fibrils formed a dense and membrane-like meshwork (figs. 1, 2, 3, 4, and 7) which was closely applied to the sinusoidal endothelium and which appeared to form the major portion of the wall of the sinusoid. The reticular fibrils did not form an

independent meshwork about the anterior lobe cells. It was not possible to determine whether the sinusoidal endothelium did or did not form a complete lining, although the endothelial cell nuclei were usually but not invariably widely spaced (figs. 2 and 8), and in sections of many sinusoids were not seen at all (figs. 1, 3, and 6). A majority of the separate fibrils in the vascular meshwork were more or less spirally directed (figs. 1, 2, 3, and 4).

Definite changes associated with advancing age were found in the sinusoidal reticulum. A thickening of the individual fibrils constituting the meshwork occurred and there was a possible increase in the numbers of fibrils (figs. 1 through 4). As a result there was a marked thickening and an increase in the density of the reticular meshwork in the walls of the vascular channels (figs. 4, 5, and 7).

Sinusoids from a 36-day-old rat are shown in figures 2 and 8. In figure 2 where a portion of a sinusoid is cut longitudinally, the fine reticular meshwork in the upper wall of the vessel is seen; the individual fibrils measured up to .6 microns in thickness. Note that a portion of this same vessel is collapsed and that a portion is also cut in cross section. This figure illustrates the extreme irregularity of the diameter and course of these vessels. Endothelial cells were more abundant in this section than usual. Reference to figure 8 will indicate that in the collapsed portions of the sinusoidal system the opposing walls of the vessel came in contact with each other.

As age advanced, the individual fibrils in the walls of the sinusoids continued to thicken, apparently at a slow rate, since in 180-day-old rats the thicker fibrils measured only .8 microns and 360-day-old rats 1 micron in thickness (figs. 1, 3, and 6), although rarely thicker fibers were seen (fig. 3). The reticular meshwork in the walls of these vessels was definitely thicker than in the younger animals (compare figs. 1, 3, and 6 with figs. 2 and 8).

In old rats (540 to 884 days) a pronounced but somewhat variable intensification of the age changes already indicated was found. There was a marked coarsening of the individual

reticular fibrils which frequently measured up to 2.5 microns in thickness (figs. 4, 5, 7, 9, and 12) and an increase in the number of fibrils (figs. 5 and 7). As a result of these changes in the reticular meshwork, the walls of the sinusoids were often markedly thickened. Although the individual reticular fibrils did become quite thickened, they showed little tendency to transform into collagen; however, a few of the thicker fibrils did appear to be transitional between reticulum and collagen. Considered as a whole, the individual fibrils were thicker and the membranous meshwork denser in the collapsed or partially collapsed sinusoids (figs. 9 and 12) than in the more open vessels (fig. 7). It is also interesting to note that complete collapse of portions of the sinusoids was much more frequently observed in the younger rats than in the old. This finding suggests an increased rigidity of the walls of the vascular channels of the old animals.

In the old rats there was a marked tendency for the fibrils in the walls of the sinusoids, especially in the outer portions, to become irregularly arranged and tangled and to send many fibrillar offshoots among the surrounding parenchymal cells (figs. 4, 5, 7, and 10). Apparently, most of these fibrils remained attached to the sinusoidal walls and they did not show any tendency to form a network about the anterior lobe cells. In figures 5, 10, and 11, three sections through the same vessel are pictured. Figure 5 shows a large lumen surrounded by a thickened irregular wall, while figure 10, which is two sections removed, shows only a section through the wall of the same channel. Figure 11 shows the succeeding section; these two thickened and irregular fibrils represent fibrillar offshoots from the wall of the vessel. Unless serial sections had been studied, it would not have been recognized that these fibrils were associated with the wall of a sinusoid.

DISCUSSION

The anterior pituitaries described above were taken from the same group of rats in which age changes in the uterus were previously studied (Wolfe et al., '40 and '42). A com-

parison of the effects of advancing age on the fibrillar material of the uterus and of the anterior pituitary brings out certain similarities and certain differences. In the uterine endometrium of young rats between 10 and 15 days of age, there is a thickening of the reticular fibrils accompanied by collagenous transformation which usually occurs when the fiber is less than 1 micron in width. This transformation process is largely completed in 90-day-old rats but throughout life most of the remaining reticulum changes into collagen and the collagenous fibers continue to become coarser, sometimes measuring up to 30 microns wide. In the anterior pituitary the pattern of age changes in the fibrillar material is different. The reticular fibrils thicken slowly throughout most of the life span and may eventually reach a diameter of 2.5 microns; yet they show little tendency to be transformed into collagen. However, we do not doubt the capacity of these fibrils to change into collagen.

It seems likely that these structural differences in the response of the fibrillar material of the uterus and of the anterior lobe to advancing age must be associated with the functional differences of the fibers in the two organs. In the uterine endometrium fibrillar material is primarily a supporting framework for the epithelial and vascular structures of the uterine mucosa although other functional capacities cannot be excluded. In the anterior lobe the reticular meshwork is an inherent part of the sinusoidal walls and may in certain portions of the sinusoidal system form the entire wall. Therefore, in the anterior pituitary the reticular meshwork must necessarily play some role in the exchange of nutritive material through the walls of the vascular channels as well as serving as a supporting tissue. In spite of the structural difference in the response of fibrillar material in the different portions of the body to advancing age, it seems highly probable that the basal process is the same. A thickening of the individual reticular fibril always occurs. The extent of this thickening and the point at which transformation into collagen occurs seem to be governed by local factors within

the organ itself. We have discussed this question in detail in a recent paper (Wolfe and Wright, '42).

Corner ('20) reported that fibroblasts are not found in the anterior lobes of cattle and thus concluded that the reticular fibrils were produced by the endothelial cells themselves. Although our studies do not offer any conclusive solution of the question of the endothelial origin of reticular fibrils, two facts should be pointed out. First, cells which could be considered fibroblasts were not encountered in the anterior lobe of the rat. Second, there was an undoubted increase of fibrillar material in the walls of the sinusoids of the increasingly older rats. Since most of the evidence points to the fact that fibrillar material is formed under cellular influence, these facts would support the view of Corner, that in certain structures of the body reticular fibrils are formed under the influence of endothelial cells. Recently, Smith and Ireland ('41) have suggested that reticular fibrils in the thymus are formed under the influence of endothelium as well as fibroblasts.

SUMMARY AND CONCLUSION

Serial sections of the anterior pituitaries of seventy-six virgin rats ranging in age from 35 to 884 days were stained by the Gömöri silver impregnation technic for the selective staining of reticular and collagenous fibrils and fibers. In the anterior pituitary of the rat the fibrillar material was almost entirely reticular and was generally found only in intimate relation with the sinusoidal capillaries. Here the reticular fibrils formed a dense meshwork which was closely applied to the sinusoidal endothelium and which formed an integral part of the sinusoidal wall. An independent reticular meshwork for the support of the anterior lobe cells was not found. Associated with advancing age there occurred a slowly progressive increase in both the number and the thickness of the reticular fibrils. The fibrillar meshwork in the sinusoidal walls became thicker and more irregular and gave rise to many offshoots of reticular fibrils which extended among the adjoining anterior lobe cells. In spite of the marked thickening

of the reticular fibrils (up to 2.5 microns), transformation into collagen occurred only infrequently.

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PLATE 1

EXPLANATION OF FIGURES

(All sections stained by the Gömöri technic.)

1 Section through a sinusoid of a 1-year-old rat, which is so cut that a portion of the lower wall of the vessel is shown in the upper part of the figure and a much smaller portion of the upper wall is shown over the lower end. Note the ends of the individual fibrils cut in cross section and the lack of endothelial cells. × 1500.

2 Section through a sinusoid of a 36-day-old rat. A portion of the upper wall of the vessel is seen, while short oblique sections of the lower wall may be seen at either end. Note that the endothelial cells are closely applied to the underlying reticular meshwork. Compare the width of the reticular fibrils pictured in this section with those in figures 1, 3, and 4. × 1500.

3 Section through a sinusoid of a 180-day-old rat. A large portion of the upper wall is shown. Note the arrangement of the fibrils in the reticular meshwork. × 2500.

4 Section through a sinusoid of a 787-day-old rat. A portion of the upper wall is shown. Note the thickening of the fibrils and the reticular offshoots away from the wall of the vessel. × 1500.

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Alice Pauline Schafer

PLATE 2

EXPLANATION OF FIGURES [2]

(All sections stained by the Gömöri technic.)

5 Longitudinal section through a sinusoid in an old rat. Note the thickened wall and the reticular offshoots into the surrounding tissues. Compare with figures 10 and 11 which are cut more laterally in the same sinusoid. × 560.

6 Longitudinal section through a sinusoid from a 180-day-old rat. The walls of this vessel are relatively thin. Compare with figures 7 and 8. Note the smaller tributory channels and that the lower wall of one can be seen. × 560.

7 Longitudinal section through a sinusoid from an 884-day-old rat. Note the irregularly thickened walls and the dense meshwork making up the lower wall of the vessel. A single endothelial cell is seen. × 560.

8 A cross section of a sinusoid from a 36-day-old rat. A portion of the channel is collapsed. Note the endothelial cells. × 880.

9 A partially collapsed sinusoid from an old rat showing a portion of the upper wall of the vessel. Note the thickened fibrils making up the reticular meshwork. × 880.

10 A section through the wall of the vessel in figure 3. Note the irregularity of the dense reticular meshwork and the many fibers extending into the surrounding tissues. × 560.

11 Fibers from the outer portion of the wall of the sinusoid pictured in figures 5 and 10. Viewed in this section the fibers apparently were lying free among the anterior lobe cells. × 560.

12 An almost completely collapsed sinusoid from an old rat. Note the extreme density of the reticular meshwork. × 880.

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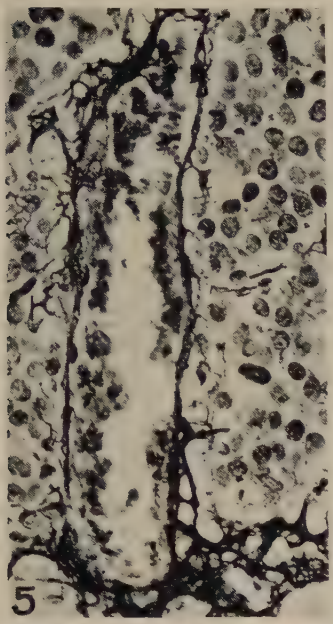
[2] We wish to thank Mr. E. G. Moore for making the photomicrographs used in this paper.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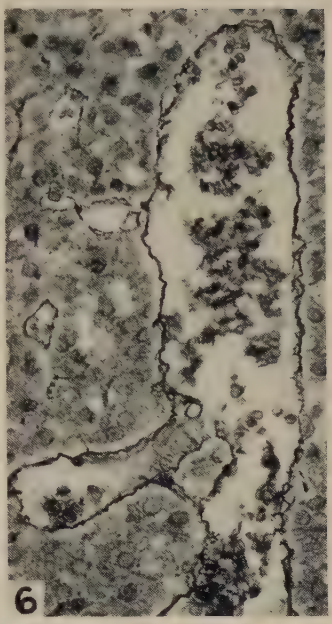
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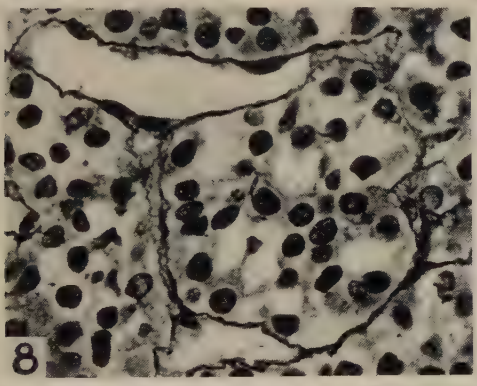
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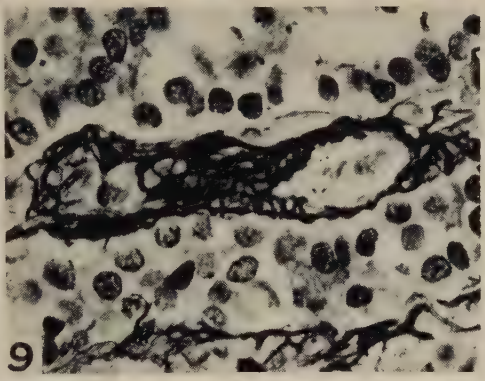
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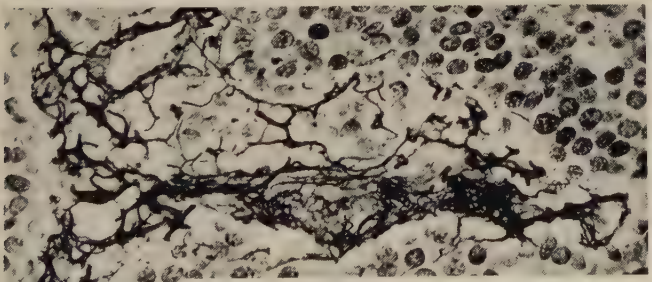

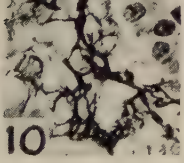
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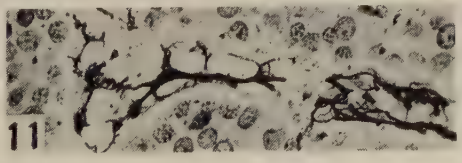
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OBSERVATIONS UPON THE STRUCTURE OF THE THYROID GLAND IN WHALES

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ONE TEXT FIGURE AND THREE PLATES (NINE FIGURES)

The object of this report is to present some observations which have been made upon the gross and microscopic structure of the thyroid gland in whales. Thyroid glands from the adult sperm whale (Physeter megalocephalus) and blue whale (Balaenoptera sibbaldii) were collected by E. M. K. Geiling, Lloyd N. Tarr and John T. Litchfield, Jr. during the summers of 1934 and 1935, through a grant-in-aid from the National Research Council; the collections were made at the whaling stations of the American Pacific Whaling Company at Rose Harbor and Naden Harbor, Queen Charlotte Islands, British Columbia, Canada. As was inevitable considering the size of the animals and the methods of catching and subsequent handling, the glands could be dissected out only after a very considerable delay. As a result, the histological fixation of the tissues is on the whole quite poor. In the case of two sperm whales, the entire thyroid gland was removed without damage and fixed in toto for gross morphological study. Ten per cent formalin was used as a routine fixative. Material from the thyroid gland of an adult "little white whale" (Delphinapterus leucas), from the Northern Atlantic Ocean, was obtained through the courtesy of Dr. Leonard F. Bélanger, of the Université de Montréal. The material was fixed in 10% formalin and in Bouin's fluid, and the histological fixation is quite satisfactory. For permanent preparations of

all tissues, blocks were embedded in either paraffin or celloidin, sectioned at varying thicknesses, and stained either with hematoxylin alone or with hematoxylin and eosin.

OBSERVATIONS

Gross structure of the thyroid gland

Sperm whale. In the sperm whale the thyroid gland typically exhibits two lateral lobes, connected superiorly by an isthmus. The appearance of a fresh specimen, viewed from its anterior aspect, is illustrated in figure 1. The fresh weight of the

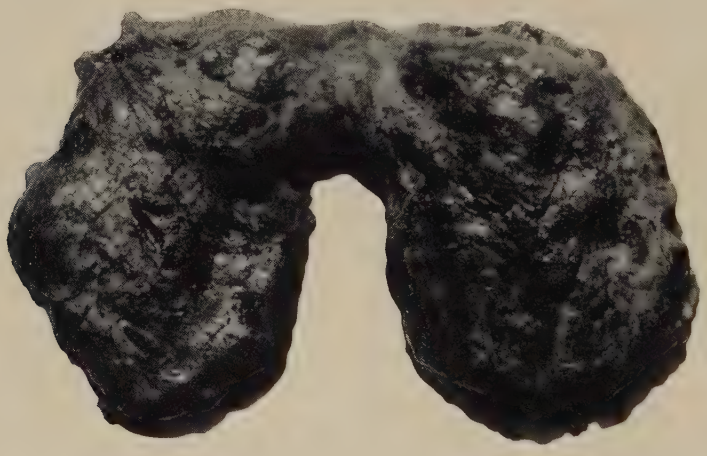
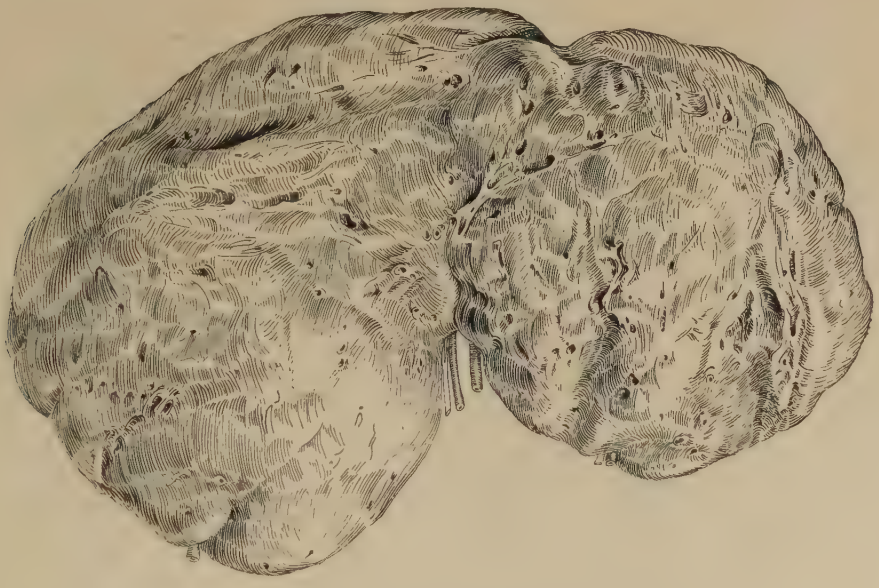


Fig. 1 Photograph of anterior aspect of fresh thyroid gland from a male sperm whale. Approximately three-tenths natural size. See text.

gland was approximately 1400 gm. Its measurements were roughly as follows: greatest transverse — 13 inches; greatest thickness — 3 inches; height of each lobe — 6½ inches; height of isthmus — 2½ inches. The appearance of a second specimen, viewed from its posterior aspect, is illustrated in figure 2. The fresh weight of this gland was approximately 1000 gm. Still a third specimen, exhibiting an unusual degree of fusion of the lateral lobes in the mid-line, is illustrated in figure 3 (viewed from its anterior aspect). This gland likewise weighed approximately 1000 gm. The two specimens illustrated in figures 2 and 3 were drawn after they had been thoroughly

2 Drawing of posterior aspect of formalin-fixed thyroid gland from a male sperm whale. $\frac{2}{5}$ natural size. See text.



3 Drawing of anterior aspect of formalin-fixed thyroid gland from a male sperm whale. The specimen exhibits an unusual degree of fusion of the lateral lobes in the mid-line. $\frac{2}{5}$ natural size. See text.

hardened in 10% formalin. The connective tissue capsule has been stripped from the gland in each instance. In the course of stripping the capsule, a careful search was made for the parathyroid glands, but without success.

Blue whale. In the blue whale, as in the sperm whale, the thyroid gland exhibits two lateral lobes and a connecting isthmus. The gland was removed as carefully and as completely as possible in two instances. The fresh weight of the specimens was approximately 3200 gm. and 3700 gm. (from a whale 77 feet long) respectively. However, it proved impossible to obtain perfect specimens because of the large size and the firm attachments of the gland. A note made by one of us (E.M.K.G.) at the time of dissection states that when the fresh thyroid gland was cut into it "gave forth a distinctive disagreeable odor, musty and penetrating." No such observation was made for the specimens obtained from the sperm whale.

Histological structure of the thyroid gland

Sperm whale. In order to survey the histological structure of the thyroid gland illustrated in figure 2, seven rather large blocks of tissue were taken from different regions of the gland, embedded in celloidin, sectioned at 8 μ, and stained with hematoxylin and eosin. As noted above, this material is quite poorly fixed, and merits only a brief description. Sections from the seven blocks yield a fairly consistent picture. There is a wide variation in follicular size: the smallest follicles are comparable with those illustrated in figure 7; the largest follicle encountered is approximately 1.3 mm. in diameter; and there are all intermediates between these two extremes, although very large follicles are present in only small numbers. In the majority of instances, follicles of considerably different sizes are found side by side, in an apparently random distribution. However, in many areas of the gland all of the follicles are of approximately the same size (small or intermediate). The follicular epithelium is on the whole cuboidal in type; on the other hand, as in mammalian thyroid glands

in general, careful search reveals all intermediates between a flattened and a low columnar epithelium. No interfollicular cells, comparable with the "parafollicular" cells of Nonidez ('33), can be identified anywhere in the gland.

The histological structure of the thyroid gland illustrated in figure 3 was surveyed in the same manner as described above, using seven rather large blocks from different regions of the gland. Here again, the fixation of the tissue is quite poor. The description given above applies so well to this gland that only the following differences need be noted: (1) very small follicles, such as are illustrated in figure 7, are on the whole more numerous; (2) very large follicles are absent; and (3) a flattened follicular epithelium, particularly in the larger follicles, is more frequently encountered.

Histological examination of other available material from the sperm whale adds nothing to what has been said above. In no instance is there evidence of any pathological process involving the thyroid gland.

Blue whale. Material is available from the thyroid gland of three adult blue whales. In the case of two of these specimens, the histological fixation of the tissue is entirely inadequate. In the case of the third specimen, on the other hand, fixation is sufficiently good to justify both description and illustration. Unfortunately, only a few blocks of this material are available, so that it has not been possible to carry out a survey of the entire gland.

As in the sperm whale, (1) there is a wide variation in follicular size; (2) in the majority of instances follicles of considerably different sizes are found side by side, in an apparently random distribution; and (3) in many areas of the gland all of the follicles are of approximately the same size (small or intermediate). In contrast to the sperm whales studied, rather large follicles are fairly numerous. The follicular epithelium is for the most part cuboidal in type, but all intermediates between a flattened and a low columnar epithelium are encountered. No interfollicular cells, com-

parable with the "parafollicular" cells of Nonidez ('33), can be identified. As in the case of the sperm whales, there is no evidence of any pathological process involving the thyroid gland.

From the block of tissue exhibiting the best fixation, serial paraffin sections were prepared, and from them three areas have been selected for photomicrography (figs. 4, 5 and 6). The description of these areas is supplied in the legends for the figures, and need not be repeated here.

Little white whale. The thyroid gland is invaded from the periphery, sometimes for a considerable distance, by a number of connective tissue septa, in which are lodged the larger vessels. These septa are quite variable in thickness, and the thickest ones are of considerable size. In some instances, particularly near the periphery, small portions of the gland (as seen in thin sections) are completely surrounded by connective tissue, giving the appearance of a "lobulated" structure. However, it seems likely that, as in man, a truly lobulated structure does not exist (Rienhoff, '30).

The follicles vary considerably in size, but no really large follicles are present. A low-power photomicrograph of the gland is reproduced in figure 8, and illustrates practically the entire range of variation in follicular size. The figure illustrates another feature of the gland, i.e., that the amount of interfollicular connective tissue is on the whole rather large. Careful search of this connective tissue reveals a complete absence of "interfollicular" cells, comparable with the "parafollicular" cells of Nonidez ('33). Where the blood vessels are naturally injected with erythrocytes, it is apparent that each follicle is invested by an enormously rich capillary bed.

Two high-power photomicrographs of the gland are reproduced in figures 9 and 10, and illustrate the general appearance and character of the follicular epithelium, as well as of the colloid, after formalin and Bouin's fixation respectively. Whereas the colloid usually exhibits a peripheral "scalloping" in Bouin's material, this effect is rarely encountered after fixation with formalin. The follicular epithelium is on the

whole cuboidal in character, and its appearance is rather remarkably consistent, particularly with regard to cell height, throughout the gland. However, as would be anticipated, a careful survey of many sections reveals a rather considerable variation, with all intermediates between a flattened and a low columnar epithelium. Here and there in the follicular epithelium one observes cells with densely eosinophilic cytoplasm, and with hyperchromatic and usually shrunken nuclei. These cells, which may occur either singly or in groups, are obviously homologous with the "colloid" cells of Langendorff (1889), which are regularly present, for example, in the adult human thyroid gland.

In unstained celloidin sections, or in sections stained lightly with hematoxylin alone, the follicular epithelial cells are observed to contain, with considerable regularity, inclusions of yellow pigment. These inclusions are quite variable in size, and some of the largest ones have a mulberry shape, apparently having been formed by the partial fusion of numerous smaller masses. They vary in color from a very pale yellow to a rather pronounced brownish-yellow. Irrespective of size, they are located predominantly in the apical and paranuclear portions of the cells; but they not infrequently extend into the basal portion, and this is particularly true in cells which contain them in unusually large numbers. This pigment is entirely analogous in appearance to that found in the thyroid gland of man and other mammals, and is presumably to be classified as wear-and-tear pigment (Abnutzungspigment) (Grafflin, '39, '42).

SUMMARY

Observations are presented upon the gross and microscopic structure of the thyroid gland in the adult sperm whale (Physeter megalocephalus) and blue whale (Balaenoptera sibbaldii), and upon the microscopic structure of the gland in the adult "little white whale" (Delphinapterus leucas). In the sperm whale and blue whale, the thyroid gland typically exhibits two lateral lobes, connected superiorly by an isthmus.

In one instance, the gland of the sperm whale has been found to exhibit an unusual degree of fusion of the lateral lobes in the mid-line. In the specimens available, the total weight of the thyroid gland is considerably higher in the blue whale than in the sperm whale.

The histological structure of the thyroid gland in whales shows no significant differences from that in the non-aquatic mammals. There is a wide variation in follicular size. In the majority of instances follicles of considerably different sizes are found side by side, in an apparently random distribution. However, in many areas of the gland all of the follicles are of approximately the same size (small or intermediate). The follicular epithelium is for the most part cuboidal in type, but all intermediates between a flattened and a low columnar epithelium are encountered. No interfollicular cells, comparable with the "parafollicular" cells of Nonidez, can be identified in any of the species investigated.

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PLATES

PLATE 2

EXPLANATION OF FIGURES

All preparations: formalin fixation, 5 μ paraffin sections, hematoxylin and eosin; reproduced at a magnification of $\times$ 60.

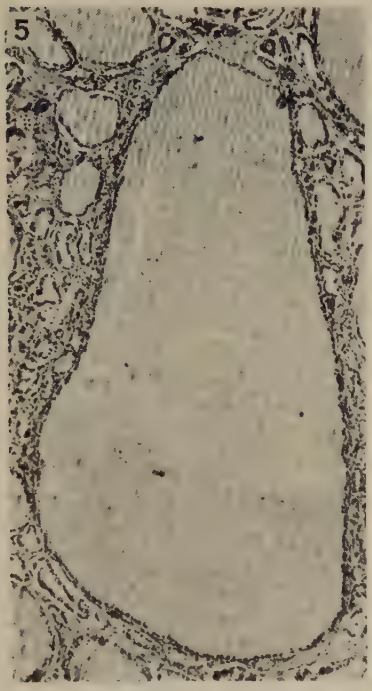
4, 5 and 6 These photomicrographs illustrate selected areas from serial sections of a block of tissue taken from the periphery of the thyroid gland of a 77-foot blue whale (Balaenoptera sibbaldii).

4 A fairly representative field, showing follicles of varying sizes; epithelium uniformly cuboidal; colloid dense and markedly eosinophilic except in very small follicles.

5 The largest follicle encountered in the sections; epithelium cuboidal, colloid dense and markedly eosinophilic.

6 The section begins at the capsule (above) and extends into the body of the gland. The large follicles at the bottom of the figure are comparable with those of figure 4. Such subcapsular accumulations of small follicles are not infrequently encountered. In some of them the colloid is quite dense, and these often exhibit a flattened epithelium. In the majority of them, however, the colloid is very dilute and stains poorly or not at all with eosin; the epithelium in such follicles is uniformly cuboidal.

7 An area of predominantly small follicles from the thyroid gland of the sperm whale (Physeter megalocephalus) illustrated in figure 3. The photograph is reproduced at the same magnification as figures 4–6 for direct comparison of follicular size. The epithelium is uniformly cuboidal; the follicular cavities usually contain delicate coagulum in small amounts, but this stains poorly, if at all, with eosin.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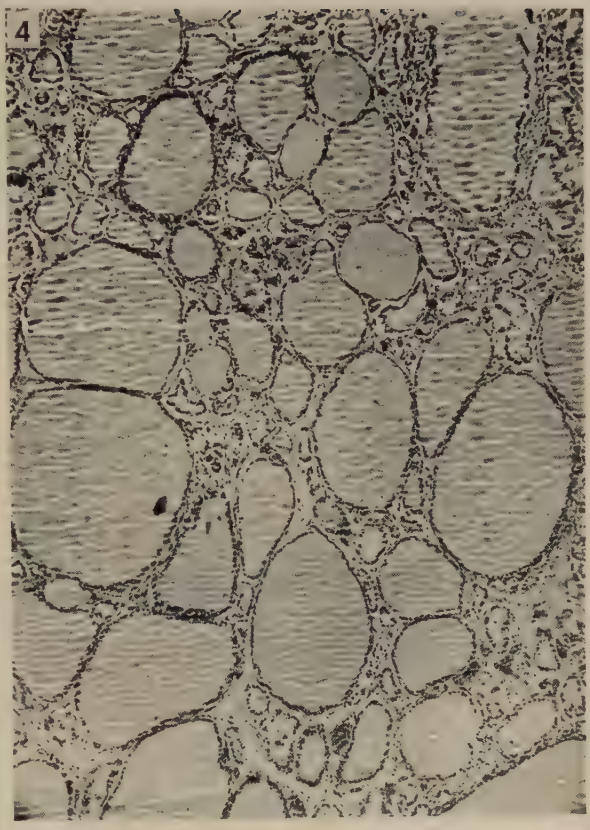
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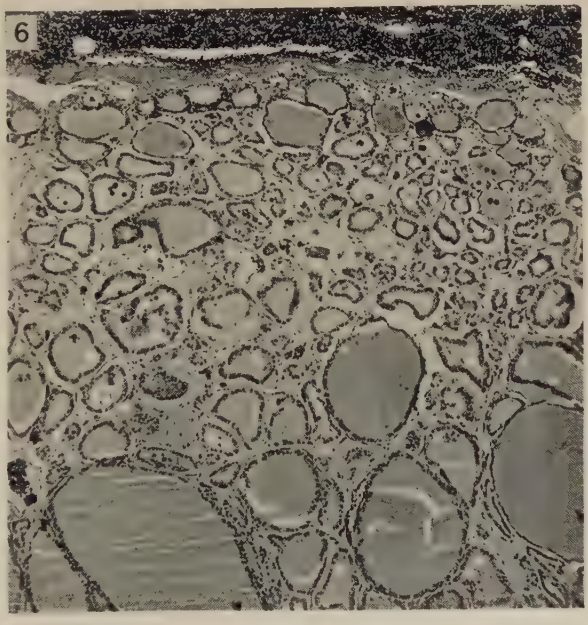
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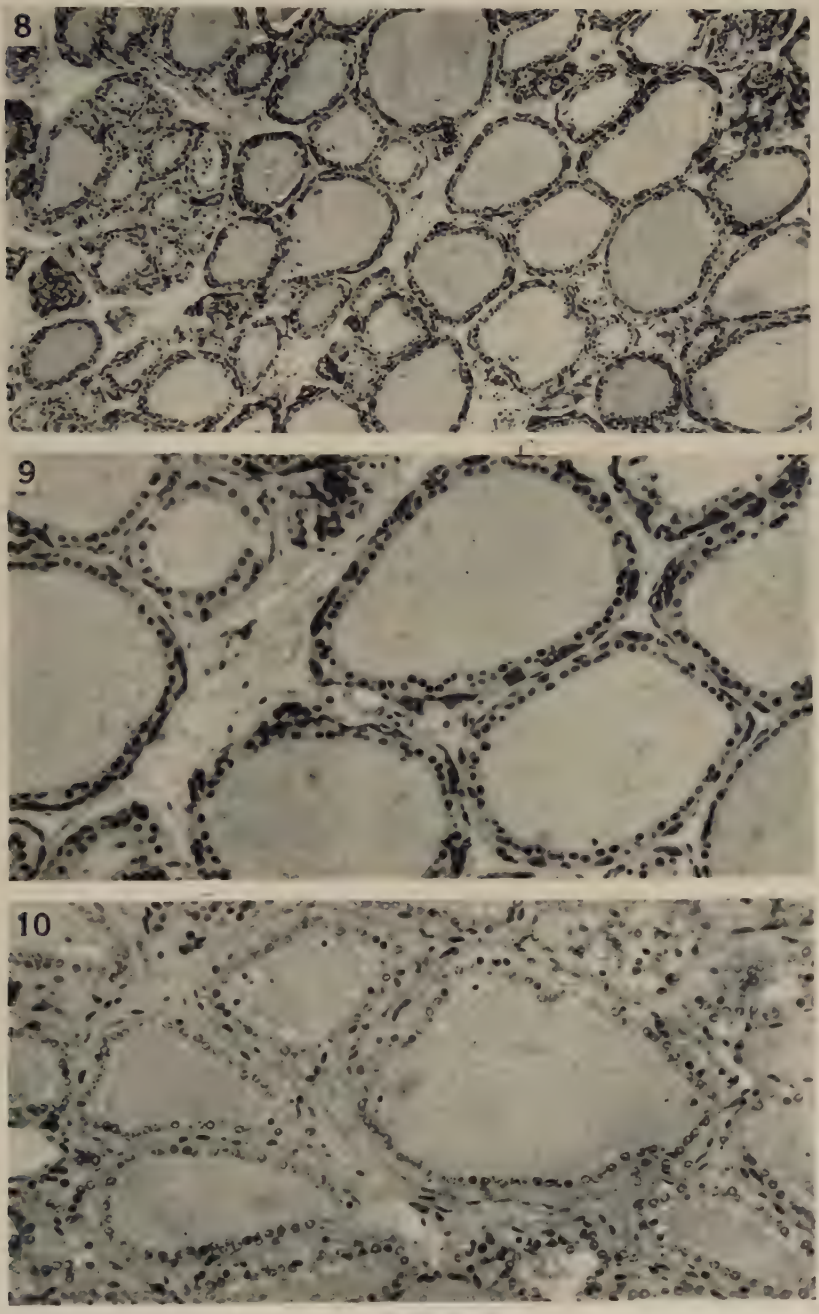
PLATE 3

EXPLANATION OF FIGURES

8, 9 and 10 Photomicrographs of the thyroid gland of the "little white whale" (Delphinapterus leucas). Celloidin sections, 4 μ, stained with hematoxylin and eosin. 8 — formalin, $\times$ 80; 9 — formalin, $\times$ 200; 10 — Bouin's, $\times$ 200. See text.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A METHOD OF MEASURING BONE GROWTH IN THE SKULL

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FIVE FIGURES

For use in an investigation we are making into the mode of growth of the skull in puppies and the influence of various factors on it, we sought a means of making exact measurements of growth at any point. It is in fact for lack of such proof that so much of the work done on these problems remains inconclusive.

The method of intermittent or remittent vital staining of bone was first tried. By madder feeding, Brash ('34) obtained some definite results in the pig skull. In puppies we found that little if any coloration was produced after 1 week of feeding, and as we wished to perform surgical experiments before the age of 3 weeks, this method was too slow for our purpose. Intraperitoneal injection of alizarin, although well tolerated by rats, proved extremely irritant to puppies. We can commend this as a reliable method to anyone who wishes to study intussusception. Neutralizing the dye, as recommended for monkeys by Schour and Hoffman ('38), and injecting it under anaesthesia, did not prevent death, which in most cases occurred within a few days. Brooks ('19) had a high mortality even in adult dogs.

In young puppies which died within a few days, all existing bone was usually stained; but the color was mostly only a pale or bluish red, and never approached the intensity of in-vitro staining with alizarin as used for cleared specimens.

Our best result was a puppy which received a single dose (30 mg. per kilogram) at the age of 18 days, and died 26 days later (fig. 1). The skull was a bright pink, intersected by conspicuous bands of new white bone at most of the sutures — but absent at the internasal, and indistinct at the sagittal. The band was 4 to 5 mm. wide at the coronal (all on the parietal side laterally); 3 to 4 mm. at the squamous (on squamosal side); 1 to 2 mm. at the malo-maxillary. A white band at the costo-chondral junctions was 8 mm. long in the fourth rib,

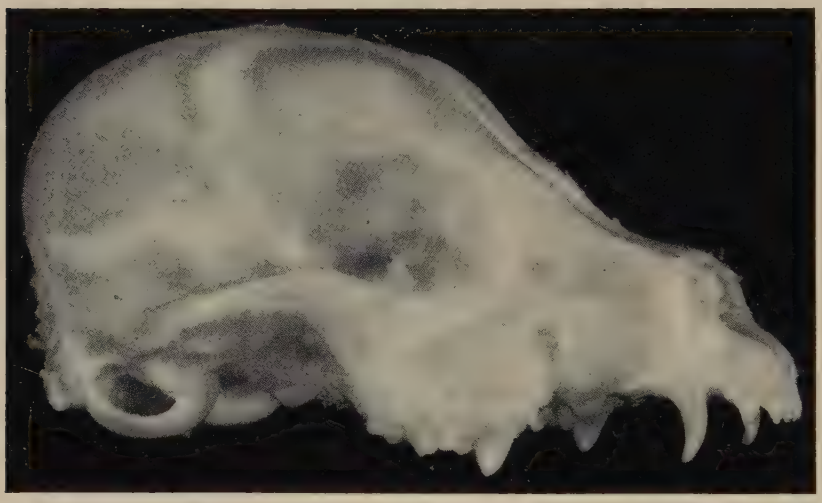

Fig. 1 Dog 17.8. Injection of alizarin red S. at 18 days old; died 26 days later. New white bone at the sutures shows as paler bands in the photograph.

10 mm. in the fifth to seventh ribs. But although the contrast between red and white bone appeared sharp on inspection, the actual demarcation at any one point was not sharp enough for exact measurement.

Gottlieb ('14) argued that it is the purpurin of madder which gives the red color to bone. A sample of this dye tried by us gave poor results. Taylor, Branion and Graham ('34) report the same experience in chickens. According to Richter ('37), the important vital dye is not purpurin itself, but purpurin-3-carboxylic acid.

Trypan blue was reported by Shipley and Macklin ('16) to show selective staining of zones of osteoblastic activity, including the suture edges, in young rabbits. Marshall ('21) injected 10 mg. doses, spaced at intervals of only 1 to 5 days, in growing rats, and described a series of separate blue lines in the dentine, one for each dose. We tried this dye in rats only, but although the suture lines after injection were strongly stained, no permanent macroscopic blue lines were produced. Histological examination of the line of ossification in long bones of new-born rats, killed 1 to 2 days after injection, showed shrinkage of the primary marrow cells, which was more marked the more blue the animal, and suggests that trypan blue is injurious to ossification. Others have reported toxicity of this dye, e.g. Taylor (loc. cit.) in young chickens.

We have now developed a simple and accurate method of measuring growth between adjacent points in the skull, on the same principle as the metal inserts used in limb bones. At the point chosen, the periosteum is reflected, and a small trephine (4 mm. diameter) is worked about half way through the bone. The disc is then cut out with a flat-pointed drill of the same diameter, care being taken not to perforate the skull. A recessed hole with sharply defined edges is thus formed. The hole is dried, and filled tightly and smoothly with bone wax, which has been mixed with sufficient carbon (blood charcoal) to make it black. The periosteal flap is replaced over the wax. The shortest distance between any two such wax inlays is measured at the time of operation and compared with subsequent measurement made immediately after the animal is killed, before any fixative has been used. There is no loosening reaction around the wax, which has been found still firmly adherent after several months, provided that the periosteum was preserved and replaced. If the region of the inlay is denuded of periosteum, surface resorption and pitting occur, and the edges of the hole become eaten away.

We have seen only two reports of similar methods applied to the skull. Von Gudden (1874) drilled small holes through the parietal and frontal bones in over twenty rabbits at 2 to 3

days old. Although no filling material was put in the holes, he stated that they remained sharply defined for 3 to 4 weeks, and even for 7 to 8 weeks on the cerebral surface. He claimed by this method to have proved interstitial growth in the parietal bone, especially near its medial and anterior borders; most of the reported increases, however, are only fractions of a millimeter. He also measured growth at the sutural edges, and found it most active at the coronal (3 to 4 mm. in 8 weeks), less at the sagittal, and least at the frontal, where it practically ceased at the fourth week.

Troitzky ('32) mentions in a footnote that by inserting silver wires in the skulls of young dogs, and taking x-ray photographs at intervals, he proved that suture growth occurs, and that interstitial growth does not; but he gives no details.

Various experiments combined with the use of wax inlays are still in progress, but some observations on normal growth have already been made. In two puppies at 23 days old, three inlays were placed in a row on each side of the coronal suture, and one other further back in the parietal bone; growth at the coronal suture was 2 to 3 mm. 3 weeks later, 4 to 5 mm. 7 weeks later; there was no interstitial growth in the parietal bone (fig. 2). In two other puppies at 25 to 28 days old, a row of three inlays was placed each side of the midline, one in the frontal and two in the parietal. In one of these puppies, killed 3 months later, the wax is no longer visible on the external surface, being smoothly covered by periosteal bone; on the cerebral aspect by bone resorption the wax has reached the surface, and in the posterior pair of inlays projects 1 to 2 mm. from the surface, but remains encased in a thin shell of bone (figs. 3 and 4). Distances between inlays cannot be measured with exactitude, but show growth at the coronal suture of 7 to 9 mm., at the frontal and sagittal of 3 to 4 mm.

The other puppy, of different breed, was killed 4 months after operation. On the external surface the inlays, which had not been well covered by periosteum, are not completely covered by bone, but are overlapped in their lateral halves

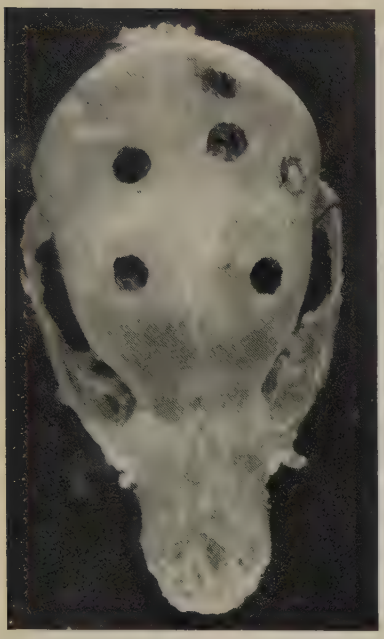

Fig. 2 Dog 21. Inlays made at 23 days old; killed 7 weeks later. Trephine holes in this case were 6 mm. in diameter. One inlay, in the postero-medial part of the left frontal, which had not been covered by periosteum, has dropped out due to bone resorption around it.

Fig. 3 Dog 26. External surface. Inlays at 28 days old; killed 3 months later. Photographed after maceration. One inlay exposed by removal of thin bone covering.

by the rising temporal ridge, 1 to 2 mm. thick (fig. 5). On the cerebral surface they are mostly hidden by pronounced intergyral ridges. Coronal suture growth measures 7 mm., frontal 3 mm., sagittal anteriorly 2 mm., posteriorly less than 1 mm.

In these two puppies, the posterior pair of inlays were placed exactly in line with the anterior border of the interparietal bone. In two other puppies a single pair of inlays were similarly placed. In all four puppies, after intervals ranging from 7 to 19 weeks, there has been no change in this relationship. All increase in length of the sagittal suture proper must therefore be due to growth of the parietal bone

at its coronal border. The respective shares of the parietal and frontal bones in growth at the medial part of the coronal suture vary considerably in different dogs, as is shown also by the varying shapes of this suture.

Fig. 4 Dog 26. Cerebral surface. Five inlays exposed by removing thin bone shell.

Fig. 5 Dog 30. Inlays at 25 days old; killed 4 months later. Skull macerated.

Although enough puppies have not been available for an adequate series at different ages, the few observations made point consistently to the conclusions that:

(i) Growth at sutural borders is a considerable factor in the expansion of the skull in puppies;

(ii) that it continues, though with decreasing intensity, at least through the first 5 months of life;

(iii) that it is minimal in the midline, but active at the coronal suture.

SUMMARY

A method is described of measuring suture growth by inlays of bone wax on the surface of the skull. Accretion of bone

on the external surface and resorption on the cerebral surface are also demonstrated by this method.

This research was made possible by the help and encouragement of Prof. B. P. Babkin, and by a grant from the Blanche E. Hutchinson Research Fund.

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A CASE OF RIGHT DUODENAL HERNIA

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TWO TEXT FIGURES AND ONE PLATE (TWO FIGURES)

The case here described occurred in the dissecting room of the Department of Anatomy in the University of Toronto. The subject was a male aged 59 years, who died of pulmonary tuberculosis.

On opening the abdomen the greater omentum was seen drawn up into the epigastric region, and below this a transparent membrane of peritoneum covering apparently the whole of the small intestine. Framing this central picture was the surrounding caecum, ascending colon, transverse colon and pelvic colon (plate 1). Inside this enveloping sac the small intestine fell as in a bag to the bottom of the pelvis, partly hiding the bladder. The sac was attached to the medial leaf of peritoneum covering the caecum and the ascending colon, to the lower leaf of the transverse meso-colon, then over the inferior pole of the left kidney to the medial side of the pelvic colon which was fixed in a high position. The final $2\frac{1}{2}$ inches of the ileum, at the level of the fourth lumbar vertebra on the right side, in the angle between the sac and the posterior abdominal wall, outside the sac, could be seen passing on to the ileo-caecal junction.

The sac was incised in the mid-line and the condition found was as follows: In the right postero-superior portion of the sac could be seen part of the duodenum apparently incorporated in the sac wall. The third and fourth parts of the duodenum were twisted and folded back upon the second part, and so intimate was the folding and blending that only care-

ful dissection could demonstrate the line of cleavage and unravel the direction of the duodenum (figs. 1 and 2). The duodenum was wholly to the right of the mid-line of the body. The stomach was opened at the pylorus, and a piece of folded wire passed through the length of the duodenum which was 8½ inches long. The first part of the duodenum passed backwards, upwards and to the right as in a normal

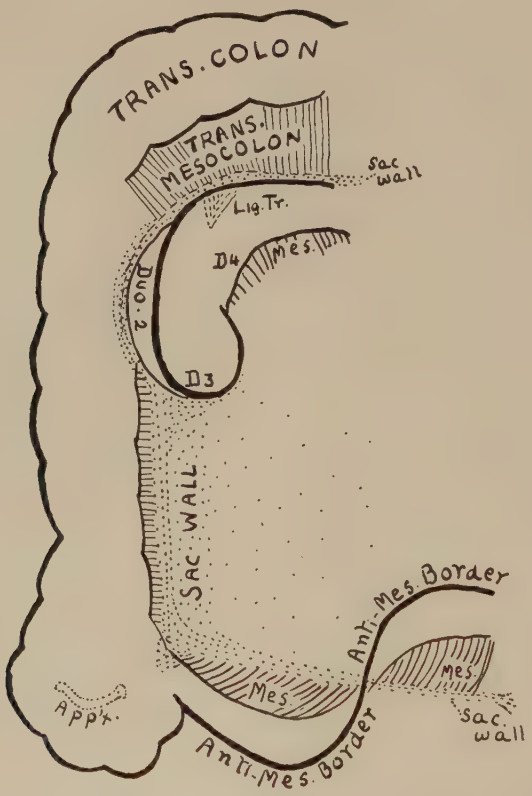

Fig. 1 The condition found on opening the sac. The upper half represents the third and fourth parts of the duodenum (D 3, D 4) twisted and folded back and adherent to the anterior surface of the second part (Duo. 2). Note the direction of the mesentery of the fourth part (Mes.) is downwards. The lower half shows the twist of the terminal part of the ileum. Appx. Retro-caecal appendix. Anti-mes. border. Anti-mesenteric border of the terminal part of the ileum. Duo. 2, D 3, D 4. The second, third and fourth parts of the duodenum. Lig. Tr. Ligament of Treitz. Mes. Mesentery of the small intestine.

subject; it then bent downwards to become the second or descending part, which was at first directed horizontally to the left for 1 inch, and then sharply over to the right and downwards for 1½ to 2 inches; it then curved upwards toward the left to the right side of the vertebral column. Then, what in a normal subject would be the third or horizontal part, together with the fourth part bent backwards, tiwsted, and its surface was applied and adherent to the anterior surface of the second part (figs. 1 and 2). It then passed to the left

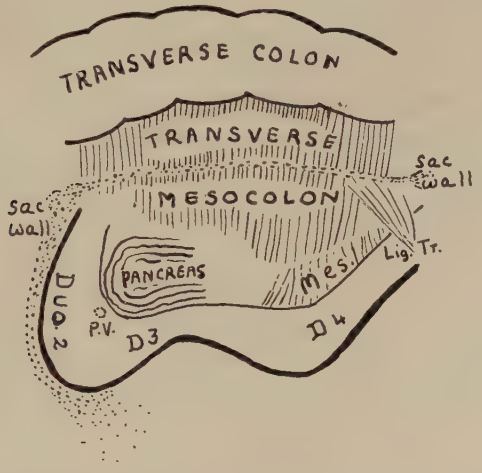

Fig. 2 To show the third and fourth parts of the duodenum (D 3, D 4) after they were dissected off the second part (Duo. 2), untwisted and laid out flat. Note the direction of the mesentery of the fourth part of the duodenum (Mes.) is upwards. Lig. Tr. Ligament of Treitz. P. V. The position of the papilla of Vater.

towards the mid-line of the vertebral column where it united with the jejunum. The duodeno-jejunal flexure was suspended from the superior wall of the sac by a definite fold which could be traced back to the postero-superior angle of the sac wall. This ligamentous fold was interpreted to be the ligament of Treitz (musculus suspensorius duodeni). From this point the jejunum became free within the sac. The sac contained the whole of the jejunum and ileum with the mesentery down to within 2½ inches of the ileo-caecal junction. The lower end of the ileum, just before leaving the sac, was twisted upon

itself in the same manner as was the duodenum. This twist resembled an S — the part of the ileum within the sac had its anti-mesenteric convex border directed upwards and to the right, with the curve running from the left to the right and downwards; while the last $2\frac{1}{2}$ inches of the ileum outside the sac had its anti-mesenteric convex border directed downwards and to the left, with the curve running from left to right first downwards then slightly upwards to the right. The distal end of the mesentery at the centre of the S naturally was twisted in like direction (fig. 1). As the ileum passed outside the sac, at the point of exit in the centre of the S, there was an oval opening half filled with a thin transparent window of peritoneal membrane ($\frac{3}{4}$ in. $\times$ $1\frac{1}{2}$ in.) filling in the aperture. This opening had a thickened supero-lateral crescentic margin, the result of traction by the bowel at the angle of exit. From the entry into the sac of the jejunum at the duodeno-jejunal junction to the exit of the ileum was a distance of $4\frac{1}{2}$ inches. This line must be considered to be the neck of the sac which had become obliterated by physiological fusion. The final 2 inches of the duodenum had a mesentery continuous with the main mesentery of the small intestine. The root of the mesentery containing the superior mesenteric artery and vein formed a curved arch dorsal to which were the aorta and lumbar vertebrae. This curved arch was attached to the posterior abdominal wall in the mid-line by very loose tenuous adhesions, which adhesions were broken down by the slightest pressure of the finger, giving admission to a retro-peritoneal space extending 2 inches to the left; the arch was 2 inches from the posterior abdominal wall at its highest point.

The superior mesenteric artery and vein were demonstrated by dissection. The artery lay to the left of the mid-line 1 inch to the left of the end of the duodenum. From this artery arose the inferior pancreatico-duodenal artery. The superior mesenteric vein lay a little to the right and in front of the artery; both vessels at the level of the duodeno-jejunal junction were wholly within the sac. No trace could be found of the right colic or ileo-colic arteries. The blood supply to the caecum

and ascending colon was by anastomosis between the right branch of the middle colic and the ascending branches of the terminal branch of the superior mesenteric artery.

The inferior mesenteric vein was demonstrated by dissection from origin to junction with the splenic. It lay in the extra-peritoneal tissue on the posterior abdominal wall behind the sac. The left colic artery was also demonstrated in its normal position from the inferior mesenteric artery, with its upper branch passing up on the posterior abdominal wall close to the inferior mesenteric vein.

In this case were found two other abnormalities: (1) a left, direct inguinal hernial opening with empty sac 1 inch long; (2) the popliteal vein bifurcated above into two veins, the anterior small vein being one-sixth the size of the posterior vein which ascended along with the sciatic nerve, and entered the upper part of the femoral vein with branches of the deep femoral.

DISCUSSION

We interpret this to be a case of right duodenal hernia arising in foetal life in a mesenterico-parietal fossa (Fossa of Waldeyer). The following is a quotation from Moynihan ('06):

"The most usual position of this fossa is in the first part of the meso-jejunum, immediately behind the superior mesenteric artery, and immediately below the duodenum. The fossa varies considerably in size. I have not infrequently seen a slight, though well-defined, bulging of the peritoneum in this situation, but I have only thrice met with a distinct fossa in adults. In seventeen embryos of less than five or six months I met with six fossae. The fossa has its orifice looking to the left, its blind extremity to the right and downward. In front it is bounded by the superior mesenteric artery, and behind by the lumbar vertebrae. The peritoneum of the left leaf of the mesentery lines the fossa, that of the right covers the blind end, and is then continued directly into the posterior parietal peritoneum. A forcible enlargement of the fossa would thus result in a tearing up of this layer of peritoneum lining the posterior abdominal wall. . . . A right duodenal hernia (*hernia mesenterico-parietalis,* Brösike) occupies in

its earlier stages the right half of the abdominal cavity. When a large size has been attained, the sac may push its way over to the left side, and finally occupy equally the two halves of the body."

In the foetal rotation of the gut, the gut involving caecum and adjacent parts of the small and large intestine rotate counter-clockwise, taking the superior mesenteric artery as an axis. In this subject under discussion a hernia occurred into the mesenterico-parietal fossa, and the small gut from jejunum to the lower part of the ileum entering the fossa rotated clockwise taking the superior mesenteric artery as an axis. The contents of the hernia pressing into the fossa enlarged to the right and upwards dorsal to the duodenum, causing the duodenum to be peeled off the posterior abdominal wall and, by continued pressure, to be folded on itself and become incorporated in the postero-superior wall of the sac. This process resulted in the direction of the mesentery of the fourth part of the duodenum being reversed from above to below the duodenum (figs. 1 and 2). The contents of the sac continued to enlarge and finally resulted in the whole of the right side and later the left side of the abdomen becoming filled. This pushing over to the left side with continued pressure resulted in physiological fusion of the posterior wall of the sac with the posterior parietal peritoneum, obliterating the orifice of the sac. This also resulted in the lower end of the ileum becoming twisted as above described. The orifice of the sac, at the time when the hernia filled only the right side of the abdomen, was bounded in front by the superior mesenteric artery and vein, and posteriorly by the vertebral column. It extended from the duodeno-jejunal junction to $2\frac{1}{2}$ inches from the ileo-caecal junction. With the hernial contents expanding to fill the left side of the abdomen, the anterior boundary wall of the orifice was pressed dorsalwards towards the vertebral column, with the formation of the loose tenuous adhesions described.

In order to demonstrate the inferior mesenteric vein and left colic artery, we incised the peritoneum of the posterior

wall of the sac on the left side. If one imagines this incision to be in the position of the original orifice of the sac, and reduces the whole of the sac contents thus rotating counterclockwise around the superior mesenteric artery, this rotation tended to unwind the twist of the duodenum and lower end of the ileum.

The duodenum was so acutely and intimately folded upon itself, that it is remarkable that our subject attained the age of 59 years without symptoms of obstruction; but a complete history is lacking.

The generally accepted view as to the origin of these herniae is that of Treitz, elaborated by Moynihan ('06), who enumerated nine fossae in the region of the junction of the duodenum and jejunum. These fossae are supposed to result from fusion folds of peritoneum during the embryonic movement of the intestinal canal. Into these fossae coils of the small intestine enter, and by continuous pressure result in herniae of various sizes. In criticising Moynihan's theories, Andrews ('23) speaks of the absurdity and grotesqueness of this whole conception, and concludes that "Duodenal hernia is a congenital anomaly due to imprisonment of the small intestine beneath the mesentery of the developing colon." For his facts and argument the original article should be consulted.

Another theory of origin recently put forward by Papez ('32) is that such a hernia has its abnormal peritoneal sac derived from the peritoneum of the normal umbilical hernia which encloses the small intestine prior to the 30-mm. stage of embryonic growth. He believes that the neck of the sac at the umbilical orifice became adherent to the duodenum by virtue of the close apposition which exists between them at this early period of development. The retracting forces which suddenly draw the intestine into the abdominal cavity, also draw in the peritoneum of the early embryonic cavity in case an adhesion happens at the umbilical orifice. This mechanism in the formation of such intra-abdominal herniae he illustrates by three figures in the text. In these figures he says, "note that the peritoneal sac which surrounds the small intestine

has been withdrawn from the umbilical cord leaving the umbilical orifice and ventral abdominal wall stripped of its original peritoneal covering.'' Certain objections to this theory are: (1) If the umbilical orifice and the ventral abdominal wall are stripped of their peritoneal covering, this should result, according to our experience in surgery, in extensive adhesions between this stripped abdominal wall and the abdominal contents; but these are not usually present. (2) If the ''actual adhesion and fusion and firm adhesion'' described by Papez ('32) occur at the umbilical orifice, one should expect certainly in many of these recorded herniae, the existence of a fibrous cord of adhesion from the umbilicus to the wall of the sac or to its contents. He quotes Lewis ('12; Keibel and Mall, p. 320, fig. 242) as having reported the presence of a peritoneal fold extending from the cephalic border of the umbilical orifice inward toward the duodenum; but such folds are prominent more by their absence than by their presence in recorded cases of duodenal herniae. (3) When the sac of the umbilical hernia has been pulled into the abdomen, the outer wall of this sac in contact with the cord has not the shining peritoneal surface covered by flattened endothelium as has its inner side, but this outer attached surface would be rough subserous areolar tissue or the embryological precursor of such tissue, and as such be subject to extensive intra-abdominal adhesions. The surface of the sac of the anomaly illustrated in his paper was apparently normal, for Papez ('32) says ''The abdominal viscera as a whole (plate 1) had a healthy exterior, presenting the smooth glistening appearance usually found in normal subjects. The peritoneal surface showed no evidence of any inflammatory process.'' He further states that the umbilical scar was normal. It would seem that this theory creates more problems than it solves.

Neither of these alternative theories explains the torsion of the small intestine found in the present case, but that propounded by Moynihan elucidates the pathological condition present.

Ziskind ('40), reported that fifty-one cases of right duodenal hernia had been recorded, and Bryon, in 1935, reported 162 cases of left duodenal hernia from a survey of the literature (Snyder, '39).

In this investigation we wish to acknowledge our indebtedness to Profs. J. C. B. Grant, J. C. Watt and W. H. Piersol for their valuable help and advice and to thank Professor Grant for permission to present this report for publication.

SUMMARY

A report is made of a case of right duodenal hernia arising in the mesenterico-parietal fossa (Waldeyer), discovered in a dissecting room subject. The sac enclosed the whole of the jejunum and the ileum down to within 2½ inches of the ileocaecal junction. Torsion of the duodenum and ileum is described, and this torsion is held to be evidence in favour of the origin of the hernia in accord with views propounded by Moynihan. Theories of origin are discussed. The presence of a direct inguinal hernial opening, and an abnormality in the veins of the leg are noted.

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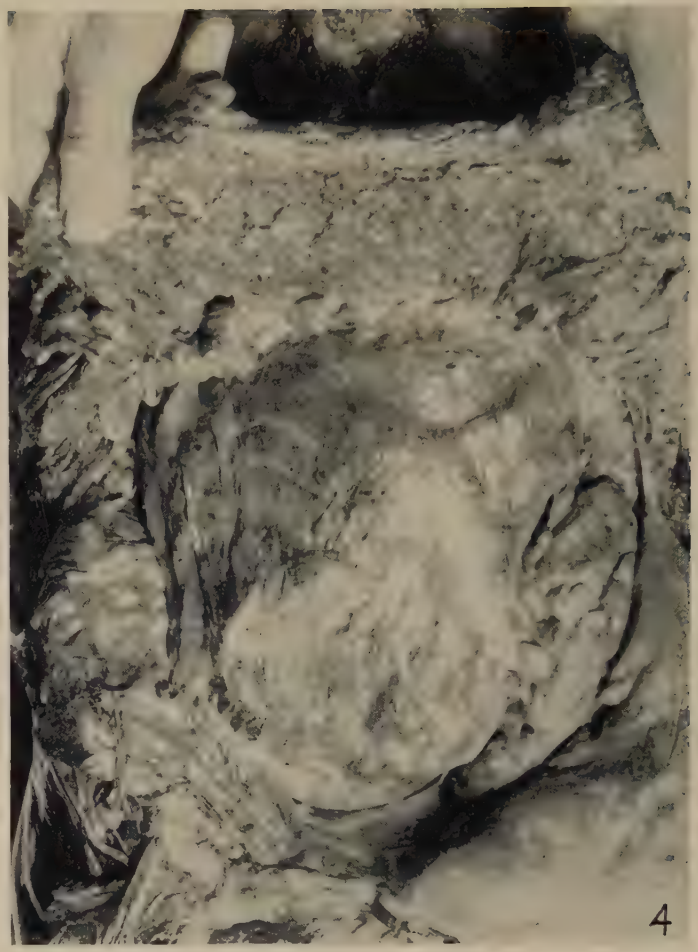
PLATE 1

EXPLANATION OF FIGURES

3 Right duodenal hernia. The sac is surrounded by the colon. The hands are holding up the greater omentum (Photograph of Artist's drawing).

4 The same as figure 3 but photograph is of the specimen.

3



4

NERVE TERMINALS ASSOCIATED WITH THE KNEE JOINT OF THE MOUSE

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THREE PLATES (FOURTEEN FIGURES)

INTRODUCTION

The macroscopic distribution of nerves to joints has been widely studied, but such histological investigations as exist are limited in scope. They have been directed toward a determination of what types of articular nerve terminals exist, but it is not always clear whether or not the endings described occur in the joints. A systematic study of the types and distribution of nerve terminals and the source of the axons which give them origin is therefore necessary if experiments are to be designed to elucidate the role played by afferent impulses from the joints in securing the coordinated activity of muscles which act over them. Some preliminary studies in this direction led to this histological investigation.

The nerve supply of joints was studied by some of the early French and German investigators. Bichat (1813), in his treatise on membranes, remarks that the ligaments are sensitive to twisting, but not to other mechanical or chemical stimulation. Subsequently Rudinger (1857), Sappey (1866), Nicoladoni (1873), Rauber (1874), Testut (1880), Krause (1874 and 1881), and Kölliker (1889) all published brief notes on the existence of nerves which followed blood vessels into joints. Rabbits and dogs were principally used and the joint capsules were prepared as thick sections or whole mounts, stained usually by the gold chloride method. Various forms of

terminal corpuscles resembling Pacinian corspuscles were observed.

Hagen-Torn (1882), in his study of the development of synovial membrane, used the gold chloride and osmic acid methods to study the formation of nerve plexuses in joint capsules and synovial membranes. Sfameni ('02) published a comprehensive account of nerves and their terminals in various types of connective tissue. Using Ruffini's gold chloride method he traced nerves along blood vessels into ligaments and synovial membranes and found numerous terminal plaques, similar to the Ruffini endings in connective tissue. He found no Pacinian corpuscles in any joint capsule. Poirier and Charpy ('11) stated that fibrous capsules and synovial membranes were rich in blood vessels and nerves, and described the occurrence of Pacinian corpuscles along articular nerves, especially in the human interphalangeal joint.

Gerneck ('32) studied the innervation of synovial membranes in human fetuses and new-borns and also provided comparative data from other vertebrates. His material was fixed in formalin, and frozen sections were cut and stained by the Bielschowsky-Gros method. He described a groundplexus composed mainly of myelinated axons and situated in the superficial part of the synovial membrane. From this plexus numerous non-myelinated axons proceeded inward. Frequently this plexus extended throughout the synovial membrane and was especially noticeable near the surfaces of the villi. Here the axons terminated as end nets, loops or knobs, and formed specialized terminals in combination with connective tissue cells.

Oda ('35) employed one of Cajal's reduced silver methods and Ranson's pyridine silver technique to prepare fetal joints of man, rabbit, and mouse. He described myelinated and non-myelinated axons in the capsule and synovial membrane, occurring especially along blood vessels. In the synovial membrane the axons were distributed near the surface in the form of a plexus. It was not obvious in what animal his observations were principally made, nor were any illustrations provided.

He stated also that nerves in the cartilage of leg joints were present in 10-day-old chicks.

The knee joint of the mouse was selected for the present study since it is small enough to be sectioned intact. All of the articular nerves can be traced from the major trunks into the joint capsule and the basic pattern of distribution of the nervous pathways determined more readily. The resemblance between the knee joint of the mouse and that of the human is so marked that the former appears to be a miniature of the latter.

MATERIALS AND METHODS

To secure adequate preparations of the joint nerves, a technique was needed which would allow nerves to be traced from the major trunks into the joint and its capsule. For this, clear-cut differentiation between nerves and surrounding tissue was necessary. Proper fixation was found to be of primary importance because the variety of ligaments and tendons closely associated with the knee joint tend to result in a differential shrinkage.

Knee joints were taken from mice ranging in age from 1 to 60 days. Of a variety of reagents employed, only formalin gave satisfactory fixation. In the younger animals the best results were obtained by immersion in 10% formalin for 1 to 2 days; in the older animals 20% formalin was employed for the same period of time.

After fixation, washing, and dehydration to 70% alcohol, decalcification was carried out by placing the specimens in 2½% nitric acid in 70% alcohol and leaving for 2 to 12 hours. They were then washed in 70% alcohol until neutral to blue litmus paper. The latter step was necessary because it was found that subsequent to decalcification, insufficient removal of nitric acid might result in poor differentiation.

In selecting the proper embedding medium it was noted that even in the youngest mice, consistently good serial sections were not obtained after paraffin embedding. Satisfactory results were, however, secured by double embedding in celloidin and paraffin. Low viscosity nitrocellulose dissolved in

amyl acetate was used for the preliminary infiltration of the block, taking care that too much celloidin did not enter the tissues. Otherwise wrinkles in the sections were difficult to avoid. After infiltration with celloidin, the blocks were hardened in chloroform for 5 to 10 minutes, cleared in xylol, and left in paraffin-xylol overnight at 37° C. before infiltrating with paraffin. The joints were cut serially at 10 microns in the sagittal plane, usually from the lateral to the medial side.

Before adopting the Bodian method of staining on the slide, joints were stained by Cajal's classical reduced silver nitrate methods and by Ranson's pyridine silver method. In these block silver methods the staining of the nerves was inconsistent, and differentiation between axons and surrounding tissue was often very poor. On the other hand Bodian's activated protargol method of staining sections on the slide gave results surpassing those of any comparable method, provided the sections were treated with 5% ammonium hydroxide in 80% alcohol prior to the protargol bath. The sections were carried from water to protargol (Winthrop) and left for 12 to 48 hours. In most of the preparations the axons stained black and were well differentiated from the surrounding tissue, which was colored reddish gray. In order to obtain such results consistently, certain precautions such as the use of chemically cleaned glassware and of double distilled water for solutions were always followed. For cytological controls, joints of mice of all ages were fixed in Zenker's fluid containing 10% neutral formalin, decalcified, double embedded in celloidin and paraffin, and stained by the Mallory-Azan method.

OBSERVATIONS

The nerve supply of the knee joint is best studied in mice about 8 days of age. This stage will first be described in some detail, followed by a brief account of earlier and later stages.

Mice 8 days old

At this age the joint is small enough so that nerves are readily traced in serial sections; yet, on the whole, the rela-

tions are those of an adult joint. The joint cavity is entirely formed; the synovial membrane contains numerous collagenous fibers and the layer of lining cells is clear and sharp; the ossification of the epiphyses is well advanced. The nerve supply of the mouse knee joint is mainly from two nerves, the femoral and the sciatic. In these preparations, it was impossible to determine whether any of the axons accompanying the popliteal artery came from the obturator nerve, as happens in man.

For convenience of description the knee joint is divided into regions. These are not sharply delimited, for as far as can be determined, the nerves entering each region participate in the formation of a plexus in the capsule. A given region is supplied mainly by the nerves entering it. The anterolateral and anteromedial regions contain those branches of the femoral nerve which descend in the quadriceps femoris muscle to reach the suprapatellar part of the synovial membrane and the articular capsule on either side of the patella. The articular branches of the sciatic nerve arise mainly from its common peroneal branch and reach the posterolateral and posteromedial regions of the joint. Some branches end in the posterior part of the capsule directly and others continue circumflexly around the joint in its capsule and near the bony attachments. The third region is the anteroinferior part of the capsule, around the attachment of the ligamentum patellae, which is supplied by recurrent branches of the peroneal nerve as well as by circumflex branches. These regions are indicated in figures 1, 2 and 3. Most of the nerves accompany blood vessels which are distributed to the same regions.

Posterolateral and posteromedial parts of the capsule. The articular nerves contain both myelinated and non-myelinated axons which branch repeatedly (fig. 4). These branches form an extensive plexus throughout the capsule. As the sections are carried medially from the lateral side, the head of the fibula appears and shortly thereafter, the tibia and femur with a long sheet of connective tissue stretching between them. This is the articular capsule and it is continuous with the peri-

chondrium at either end. In this plane the plexus of axons is clearly seen. They run in every direction in both capsule and perichondrium. The larger axons branch into smaller ones which in turn subdivide at nodal swellings (fig. 8). Most axons become reduced in size as the result of branching so that they ultimately pass beyond the range of visibility. Varicosities appear upon them, but none appears to have specialized endings. In the deeper parts of the capsule large numbers of articular blood vessels appear in longitudinal section. The axons are collected into bundles which lie along the larger arteries and veins and can be traced posteriorly to the popliteal space and superiorly to the thigh. These nerve bundles lie in the loose connective tissue around the vessels and during part of their course may lie within the adventitia. They follow the course of the vessels closely and branch as the vessels branch. The twigs of the nerve bundles become progressively smaller as the vessels branch and eventually form a fine plexus in the adventitia of the arterioles. The fiber bundles of this adventitial plexus pursue tortuous courses and anastomose with one another across the vessel at various angles. In those sections showing arterioles cut longitudinally, tiny axons with varicosities can be seen lying along the muscle coat. Again no specialized terminals are present; the axons simply disappear and their exact relation to the smooth muscle cells cannot be determined. Other small axons lie in the loose connective tissue closely surrounding the arterioles (fig. 5).

The irregular course of all these axons contrasts sharply with similar fibers accompanying arterioles through the bone marrow. Here such axons pursue remarkably long, straight courses, so that often considerable lengths of individual axons appear in one section, sometimes continuing as far as the zone of ossification.

As the sections are carried medially from the area of blood vessels in the capsule, the joint cavity appears, and the capsule is seen only in the anterior and posterior parts of the joint (fig. 2). In the superior and inferior parts of the posterior capsule the articular nerves now appear in cross section and

may be traced from the capsule to the common peroneal nerve. The articular nerves run almost parallel to the collagenous fibers. The nerve plexus formed by the component fibers is extremely difficult to identify for almost all of the axons are cut transversely, and the smallest ones appear only as dots. Not until they deviate from this course can they be identified. As the articular nerves are followed, branches come off at almost right angles and course toward the synovial membrane in a tortuous manner. These branches may or may not accompany blood vessels. If they do not, the axons branch repeatedly in the connective tissue and the ultimate terminations take several forms. If an axon is traced toward the synovial membrane, it diminishes in size and may disappear between one section and the next. Occasionally an axon is observed to end either between the cells lining the surface or but a short distance below them. Such axons may have slight terminal swellings (fig. 10), which appear to be free endings. A small axon, or a branch of a larger one, may form an anastomosing network which has the appearance of a tangled skein lying in the connective tissue (fig. 13).

Small synovial villi, or fringes thereof, frequently project into the joint cavity. These possess a core of blood vessels and accompanying nerves which take an irregular course toward the synovial surface. The axons lie in the adventitia of the vessels and as the latter approach the synovial surface, the axons may be separated from the joint cavity by but a single layer of lining cells (fig. 6). Those axons which do not accompany blood vessels branch frequently in the connective tissue and end without any specialized form other than an occasional cluster of anastomosing twigs.

Great numbers of blood vessels and nerves enter the posterior part of the capsule, especially near the midline. These are derived from the sciatic nerve and arise mainly from its common peroneal branch. Some are the articular nerves whose course and distribution to the lateral part of the capsule have already been described. The remaining nerves are distributed to the capsule and synovial membrane near their

points of entrance. For the most part they pursue the same winding, irregular course toward the synovial membrane, branching repeatedly.

The largest myelinated axons which enter the capsule form specialized endings encapsulated by connective tissue. Before they terminate, collaterals may proceed to neighboring blood vessels. Such axons may form a thickened but coiled and tortuous terminal surrounded by one or two layers of longitudinally arranged connective tissue cells (fig. 14). These terminations may lie close to the synovial surface. Or again, the terminations may suddenly occur by the breaking up of the axon into a number of fine, thread-like fibrils which anastomose to form glomerular or loop endings surrounded by rather closely packed connective tissue cells. All such encapsulated special endings are infrequent when compared with the relatively vast numbers of axons which appear to end freely along blood vessels. Encapsulated terminations were not found in the anterior part of the capsule, in the infrapatellar fat pad, or in the cruciate ligaments. No endings of any kind were found in the cruciate ligaments. Vessels and nerves were frequently observed to enter them but when traced were found to pass into either the tibial or the femoral epiphysis. If any axons end in the cruciate ligaments the Bodian stain did not reveal them.

Anterolateral and anteromedial parts of the capsule. Branches of the femoral nerve are prominent in the capsule on either side of the patella (fig. 7). They descend almost as far as the attachment of the ligamentum patellae. Axons leave at almost right angles to enter into the formation of the plexus which was described in the posterolateral part of the capsule. This plexus is also present in the suprapatellar part of the synovial membrane at its attachment to the femur. Axons also accompany the blood vessels which penetrate the femoral epiphysis and can be traced through the cartilage as far as the vessels appear to pass. A large proportion of the axons descending from the thigh are distributed in this manner to the femoral epiphysis.

More inferiorly, axons leave the capsular branches of the femoral nerve to enter the infrapatellar fat pad. This pad is composed almost entirely of fat and has a very thin layer of lining cells next to the joint cavity. The only nerves found in it are those accompanying blood vessels, excepting a few axons which end in or near the synovial surface. No encapsulated endings were observed. As in the synovial villi, the axons lie in the adventitia of the vessels and may be separated from the joint cavity by only the synovial lining.

Anteroinferior part of the capsule. This area around the attachment of the ligamentum patellae is supplied by recurrent branches of the common peroneal nerve which ascend from the anterior part of the leg with accompanying blood vessels. No specialized terminals were observed in this region. A few axons reached the infrapatellar fat pad and others accompanied blood vessels into the tibial epiphysis.

Extracapsular terminations. Not within the capsule, though certainly functionally associated with the knee joint, are the sensory endings of nerves in neighboring tendons and muscles. Prominent neurotendinous endings are found in almost all nearby tendons. An extremely thick, myelinated axon enters such a tendon, ensheathed by parallel-lying cells which are presumably of fibroblastic or neurilemmal origin (fig. 9). This axon breaks up into smaller branches which distribute themselves throughout the tendon. Occasionally one of these branches leaves the tendon to form a neuromuscular spindle in the attached muscle. With the Bodian stain the connective tissue capsules of the spindles are indistinct, but the thick axons stain clearly. The spindles in question are usually formed of two small, thin muscle fibers around which the numerous branches of the axon entwine (fig. 12). Two axons were observed which previous to forming neurotendinous endings gave rise to branches which entered the posterior part of the articular capsule and formed encapsulated endings.

It should be noted here that many nerves enter the periosteum of the fibula, just superior to its fusion with the tibia. Axons in the periosteum terminate in sudden expansions and

around their ends several rows of large, oval, pale nuclei are collected, between which a slight amount of collagen is visible (fig. 11). These nuclei are separated from the axons by marked clear zones. Such encapsulated endings are oval in shape and their long axes are in the same plane as that of the fibula. Presumably these are developing Pacinian corpuscles, and they were not observed elsewhere in the thigh or leg. This observation is in agreement with that of Miskolczy ('26). No specialized endings approaching these corpuscles in complexity of structure were observed in the articular capsule of the knee joint.

Mice 1 to 4 days old

At birth, the distribution of nerves to the knee joint and their manner of terminating is almost the same as at 8 days. The joint resembles a fetal joint. The joint cavity is not always complete, and the synovial membrane and articular capsule are composed of a very loose connective tissue. Ossification of the epiphyses is not apparent until the fourth day. Consequently, until then, no nerves or blood vessels enter the epiphyses. The Pacinian corpuscles in the fibular periosteum are small and fewer nuclei surround the axon terminals.

Mice 14 to 60 days old

After 2 weeks, growth and maturation of the joint continue. The connective tissue of the capsule becomes denser and the amount of fat in the capsule increases. By 30 days, bone is present beneath the articular cartilages. The distribution and termination of nerves does not differ from that in the younger mice, but the increasing density of the connective tissue makes it more difficult to trace many of the axons, especially those cut transversely. The synovial villi are longer and very well vascularized. Some of the myelinated axons entering the joint capsule appear to have increased in size, but measurements have not been made. The Pacinian corpuscles in the fibular periosteum are larger. Connective tissue

lamellae are present and their cells are thin and flat. The inner bulb spaces are well marked and the axons entering them are surrounded by numbers of irregularly grouped, large, oval nuclei. The encapsulated endings in the joint capsules are neither more numerous nor more complicated than in the younger mice.

DISCUSSION

The majority of the axons entering the knee joint accompany blood vessels, and consequently this group must contain those serving a vasomotor function for the articular capsule itself and for the femoral and tibial epiphyses. However, in addition to the autonomic post-ganglionic axons comprising the vasomotor supply, this group may also include pain endings which terminate freely about the blood vessels. The relative proportions of the two groups could only be determined by comparing the proportions of those axons which remain in deafferented and in sympathectomized limbs.

It is well-known that a joint may be acutely painful either as a result of direct trauma or of inflammation. The synovial membranes of knee joints have been electrically stimulated with the result of accentuated respiratory and cardiovascular reflexes which are known to be associated with painful stimuli (Ranson and Billingsley, '16). The reflexogenic areas for the respiratory and cardiovascular reflexes were situated in the lateral and medial surfaces of the knee joints of rabbits (Raszeja and Billewicz-Stankiewicz, '34) where the present work demonstrates that the plexus is well marked and where free nerve endings are found. By inference, then, it might be expected that free nerve endings are associated with painful stimuli.

All the specialized encapsulated endings are simple in form and are found in the posterolateral and posteromedial parts of the capsule. From their positions one might expect them to be stimulated by pressure produced by flexion of the knee joint. It is uncertain what purpose such stimulation serves, since joint flexion would affect neurotendinous endings in the

muscles producing the movement. Sensory endings in the quadriceps femoris would also be subjected to stretch during the movement, though this would be of a more passive rather than of an active nature.

It is also worth noting again in this connection that no Pacinian corpuscles or anything approaching them in complexity of structure were found in the joint capsules, although the statement appears often, especially in the standard texts, that they are common in joint capsules. At least in the mouse they are found only in the fibular periosteum.

It is planned to continue this study in the joints of larger mammals in order to lay the foundation for experimental investigation of the nervous pathways to joints.

SUMMARY

Preliminary studies of nerve terminations in mammalian articular capsules are reported. Bodian's activated protargol method for staining peripheral nerve terminations was employed. Knee joints of mice varying in age from 1 to 60 days were fixed in 10 to 20% formalin, decalcified, double embedded in celloidin and paraffin, and serially sectioned at 10 microns. Sections were treated with ammoniated alcohol for 24 hours, followed by double impregnation with 1% protargol and 4 to 6% metallic copper for 48 hours. The remaining steps were essentially those described by Bodian. All types of nerve terminations stain black by this method, sharply contrasting with the reddish-gray surrounding tissue.

Nerves were traced from the major trunks into the articular capsules. In general, they closely accompanied the articular blood vessels. Entering the synovial membrane, some of the axons, presumably vasomotor, continued with the blood vessels. Other axons had apparently free endings in the connective tissue. Still others formed specialized terminals in conjunction with connective tissue. Pacinian corpuscles were not found within or associated with the articular capsule, but commonly occurred in the fibular periosteum, near articular or ligamentous attachments.

ACKNOWLEDGMENTS

I wish to express my appreciation to Prof. E. V. Cowdry for the many privileges which he has extended me and the facilities which he has placed at my disposal, and to Dr. James L. O'Leary for his valuable criticisms and suggestions made in the course of this study.

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PLATE 1

EXPLANATION OF FIGURES

1 Outline drawing of a section through the lateral side of the knee joint to indicate the lateral side of the articular capsule.

2 Outline drawing of a section through the lateral meniscus. Only the anterior and posterior parts of the capsule are present in this plane.

3 Outline drawing of a section through the anterior cruciate ligament and infrapatellar fat pad. The posterior part of the capsule is the point of entrance of many of the articular vessels and nerves. The areas of entrance of the epiphyseal vessels and nerves are shown.

1, femur; 2, fibula; 3, tibia; 4, patella; 5, common peroneal nerve; 6, lateral fabella; 7, lateral meniscus; 8, anterior cruciate ligament.

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2.

3.

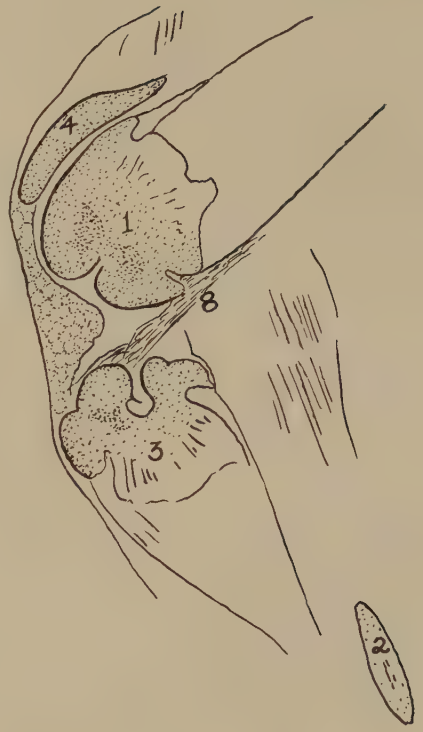


PLATE 2

EXPLANATION OF FIGURES

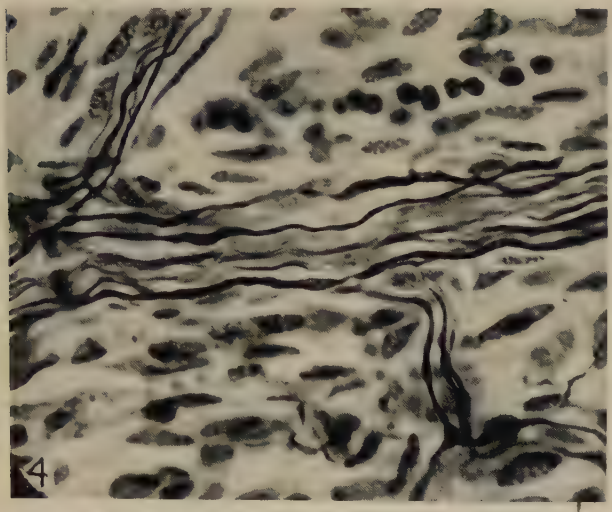
4 Bundles of axons in the lateral part of the knee joint capsule. × 700.

5 Two axons lying in the connective tissue of the vascular part of the capsule. Note the numerous capillaries. × 700.

6 A synovial villus. The axons lie between a large vein and the joint cavity. × 700.

7 Longitudinal section of the anterolateral part of the capsule. Axons from the femoral nerve form a descending bundle. × 700.

8 Two axons in the capsular plexus. Note the varicosities and the branch at a nodal swelling. × 700.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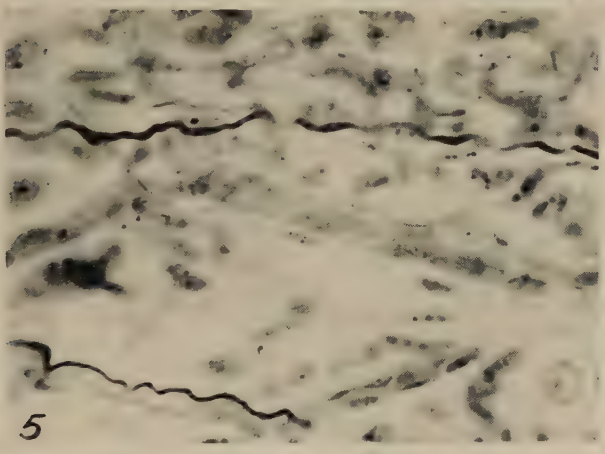
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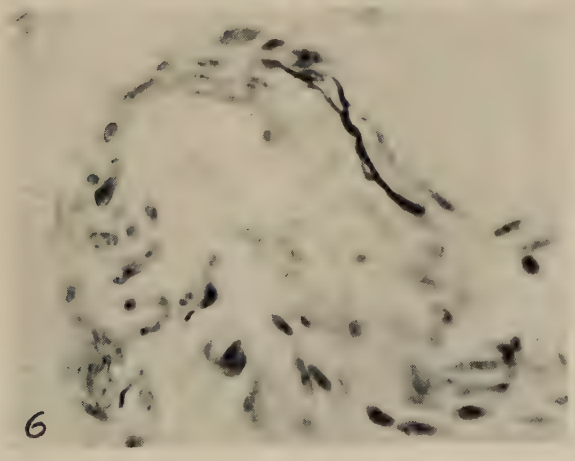
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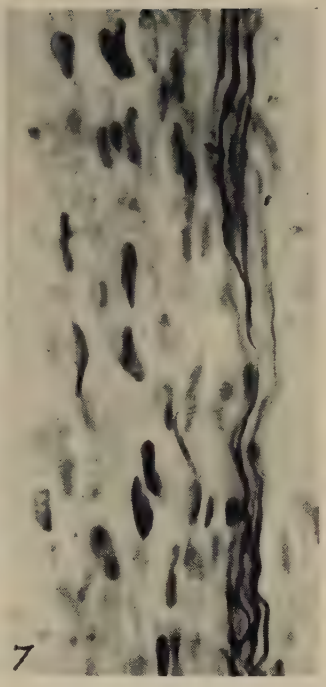
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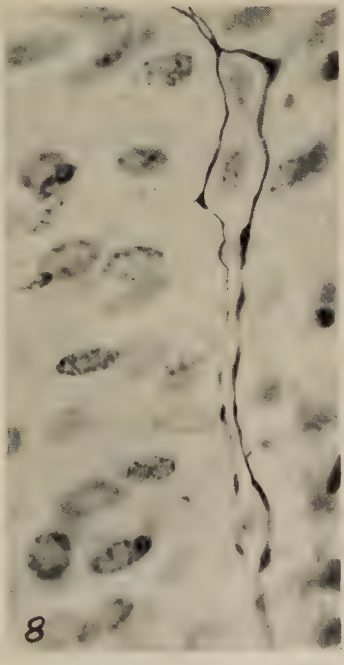
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PLATE 3

EXPLANATION OF FIGURES

9 Entrance of a large myelinated axon into the gastrocnemius tendon just before breaking up into terminal branches. × 700.

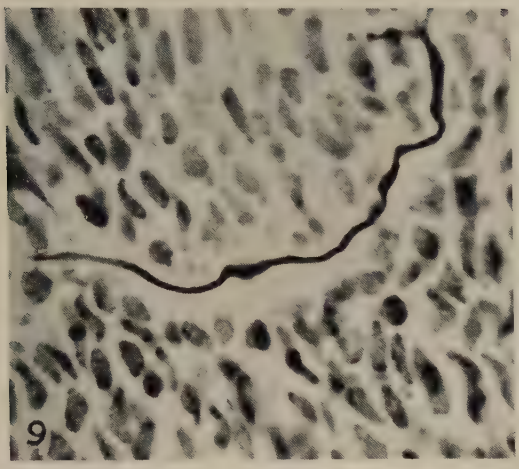
10 Terminal swelling of a non-myelinated axon a short distance from the synovial surface. × 700.

11 A Pacinian corpuscle in the fibular periosteum. The space between the axon and surrounding cells is well marked. × 700.

12 A neuromuscular spindle. Note the absence of striations within the spindle. × 700.

13 A non-myelinated axon breaking up into a group of anastomosing fibrils. A few nuclei of connective tissue cells are near. × 700.

14 A specialized encapsulated termination in the posterior part of the capsule. Note that it is so coiled that parts of the axon are not in focus. This is near the joint cavity. × 700.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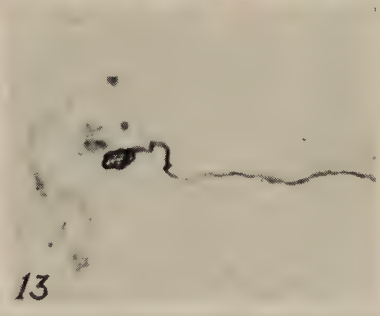
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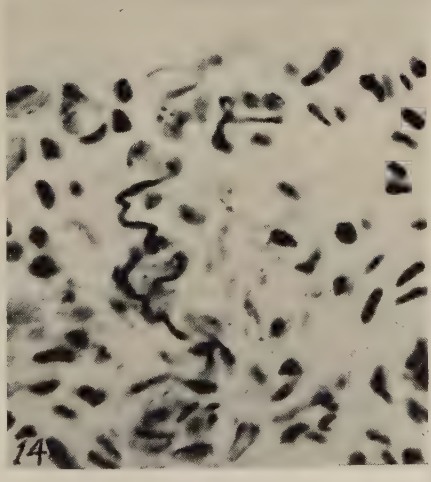
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THE PERIOVARIAL SAC IN THE ALBINO RAT [1]

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ONE TEXT FIGURE AND ONE PLATE (ONE FIGURE)

The question of the presence or absence and the possible functions of an "ovarian sac" (Robinson, 1887) has been the subject of several morphological and at least two embryological studies.

Although Emmert and Burgatzky (1818) had previously described a nearly closed capsule in the bat, Treviranus (1825) seems to have been the first to describe a completely enclosed ovary (in Mustela foina) and to establish the relationship between the oviduct and the capsule.

In 1887 Robinson made a study of the peritoneal relations of the mammalian ovary and classified the various types of ovarian capsules according to the extent of closure. Unaware of earlier work (Treviranus, 1825; Weber, 1826), Robinson described what he believed to be a new type of capsule: one completely shut off from the peritoneal cavity and connecting with the exterior through the oviduct. Such a condition he found in Mus musculus and Mus decumanus. To make sure no orifice was overlooked,

> A series of animals was taken and through the uterus and Fallopian tubes injections, in some cases of colored fluids, in others of air, were forced. These injections entered and distended the ovarian sacs, but in no case escaped from them into the peritoneal cavity. Thus it was definitely proved that the only communication of the sac was with the uterus through the Fallopian tube, and that there was no opening from the sac into the peritoneum (p. 176).

[1] A part of the dissertation, presented for the degree of Doctor of Philosophy in Yale University. It is a pleasure to acknowledge the generous counsel and encouragement of Professor J. S. Nicholas, under whom the work was done.

Serial sections were made and were interpreted as showing a completely enclosed space. ". . . the rat and the mouse present us with a . . . peritoneal ovarial sac . . . the cavity of which is completely shut off from the abdominal cavity, and whose only communication is with the uterus, through the Fallopian tube" (p. 177).

Zuckerkandl (1897) in his classical study, "Zur vergleichende Anatomie der Ovarialtaschen," mentions the four types suggested by Robinson but inexplicably misses the fifth type (the closed sac of the Muridae) for which the paper was written! Zuckerkandl himself did not study the Muridae.

Gerhardt ('05), failing to mention the work of Robinson or Zuckerkandl, describes the condition in Mus musculus as follows: "Dieser Raum [the periovarial space] kommuniziert durch keinerlei Oeffnung mit der Bauchhöhle" (p. 678). Fischel ('14), in describing the condition in Mus decumanus, says: ". . . dieses [the infundibulum tubae] liegt . . . innerhalb der allseits geschlossenen Ovarialkapsel . . ." (p. 581). Grosser ('19) refers to the capsule as being closed, citing Gerhardt as his authority.

Sobotta ('17) states, ". . . bei den Muriden das Mesenterium tubae (Mesosalpinx) eine vollkommen gegen die Peritonealhöhle abgeschlossene Ovarialkapsel erzeught . . ." (p. 366). He notes that preceding ovulation the capsule is distended with fluid and understandably concludes that this could occur only if the enclosing capsule were anatomically complete.

Both Allen ('22) and Long and Evans ('22) speak of the ovarian sacs as closed; the latter, apparently unaware of the earlier observations of Fischel ('14) and Sobotta (1895, '14, '17) concerning the periovarial fluid, have the following to say: "Another feature characteristic of ovulation never before to our knowledge mentioned in the literature is the accumulation of fluid in the periovarial space, causing a distention of the periovarial membrane which, in pathological cases, may be very great . . ." (p. 32). And again, that "The periovarial

space has no connection with the peritoneal cavity . . . [is] shown by the distention of the membrane by a characteristic fluid at the time of ovulation'' (pp. 15–16).

Of the mouse, Allen says, ''The ovaries . . . are completely surrounded by closed sacs of peritoneum from which the openings of the two oviducts lead.'' This anatomical character, Allen concludes, is of particular significance, because ''. . . the number of ova in the tubes and periovarian sacs is always the number ovulated, since none can escape into the peritoneal cavity.''

A similar conclusion is drawn by Novak and Eisinger ('26) and Kelly ('39).

Agduhr ('27), in a work very generally overlooked on the development of the bursa ovarica and uterine tubes of the mouse, seems to contribute the final evidence — the embryological closing of the bursa. Describing the condition in the 9-day-old, 34-mm. embryo, he says: ''The ovarial bursa is entirely closed; as far as one can judge the closing of the bursa has taken place here also caudal to the ovary through coalescence between parts of mesotubarium inferius.'' Kellogg ('41) states that an opening from the capsule into the abdominal cavity closes on about the fourth day after birth.

Kingery ('17) found the capsule complete in ''rather more than half'' of the white mice examined; in the remainder ''there was an opening into the peritoneal cavity'' (p. 271).

The significance of the capsule has been variously interpreted. It has been repeatedly shown that its removal does not cause sterility, although fecundity is considerably lowered (Novak and Eisinger, '26; Neugebauer, '35, quoted in Hartman, '39; Kelly, '39). Agduhr ('27) suggests that the periovarial space may serve as a storage place for spermatozoa. A more recent and highly original function ascribed to the capsule is that proposed by Grumbrecht and Losser ('39); namely, that the capsule serves to force follicular fluid, liberated at ovulation, to pass into the uterus, where the estrogens will act upon the uterus. The best known of the hypothetical func-

tions attributed to the ovarian capsule is that postulated by Fischel ('14). He believed that contraction of the smooth muscle, which he found in the mesentery forming the capsule, compressed the periovarial fluid, and that it was in this manner that the ova were transferred from the ovary to the oviduct.

OBSERVATIONS

During an investigation of the mechanism of egg transport through the oviduct the ovary and capsule were carefully examined with the idea of measuring, if possible, any pressure developed by muscular activity of the oviduct or capsule, and during this examination it was found that in the albino rats of the Osborn Zoological Laboratory colony the periovarial space actually communicates with the peritoneal cavity by a small but definite opening located on the ventro-medial (antimesometrial) side of the bursa. It is apparently the result of incomplete fusion of the mesotubarium inferius in the region of the ligamentum ovarii proprium. Beginning as a groove between muscle bundles of the cornu tip, it extends as a slit into the periovarial space, opening at the hilus of the ovary next to the ostium tubae (fig. 2).

It was first assumed that this incompletely closed ovarial bursa was an anomaly peculiar to the particular strain of rats. However, a similar condition was found in the house mouse and in crosses between the albino rat and the wild rat. An examination of the figures of other authors (especially Fischel, '14) confirms the belief that the condition existed in their material. The fact that the opening is of the nature of a canal and that it runs on an angle makes it impossible to demonstrate the opening in a single microscopic section; only by tracing sections serially can the opening be demonstrated.

What, then, of the experiments of Robinson and the observations of Sobotta, Fischel, and Long and Evans concerning the extension of the periovarial membrane with fluid? It will be recalled that Robinson injected fluid through the uterine cornu

and distended the bursa in this manner. This was taken as proof of a completely closed sac. A similar experiment was performed, except that the fluid was injected directly into the tube, through the utero-tubal opening. The result was much the same as that described by Robinson: the periovarial sac was slowly distended and the colored fluid was clearly visible through the thin wall of the capsule. After a time, in a few cases, some of the colored fluid appeared outside the capsule. Only a relatively small amount appeared and might easily have escaped notice but for the fact that the organs were surrounded by absorbent cotton pads soaked in salt solution; the cotton fibers readily adsorbed the nile blue sulfate, used for coloring the solution, becoming bright blue. Minute quantities of the escaping fluid were thus readily detected.

Obviously the injected fluid was entering at a more rapid rate than it was leaving. However, the normal secretion into the periovarial space, noted by Sobotta and others, cannot be presumed to appear suddenly, as though injected; it must be delivered slowly as is normally true of any secretion. Furthermore, the opening is not minute but is anatomically of sufficient size to release fluid at a rapid rate. But that it does not normally do so is clearly shown by the swollen condition of the capsule typical of oestrus. Some mechanism is apparently at work keeping the opening from functioning as completely as one would expect from its absolute size. It may be noted that folds of the oviduct cover the region of the opening but could not conceivably effectively close it. Nor could the smooth muscle fibers bordering the opening in any way act as a sphincter. Examination of living and fixed material (dissections and cytological preparations) has led to the following conclusion. The fimbria of the oviduct are so situated as to obstruct partially the opening from inside the capsule. Isolated fimbria may be found extending along the Musculus infundibulum tubae for some distance (fig. 2). It seems probable that contraction of this smooth muscle bundle, as suggested by Fischel ('14), together with a hyperaemia of the tubal tissues (Andersen, '27; Wislocki and Dempsey, '39) and

an increase in fluid within the capsule, causes the fimbriated tip to be brought over the opening so as nearly to seal it. In some cases fimbria — more rarely follicles — have been seen protruding from the opening into the abdominal cavity.

Fischel ('14), finding muscle fibers in the periovarial membrane (Musculus mesenterii tubae) and others connecting to the fimbriated end of the tube (Musculus infundibuli tubae), suggested that the transport of eggs from ovary to tube is accomplished somewhat as follows: fluid is secreted into the periovarial space, its purpose being to surround the recently ovulated eggs, to protect them, and to carry them into the ampulla. This is effected by a contraction of the Musculus mesenterii tubae and of the Musculus infundibuli tubae, the former increasing internal pressure, the latter opening the ostium. The pressure being lower in the tube, the fluid with the ova is carried into the ampulla, which, it was noted, always carries eggs surrounded by a quantity of fluid some hours after ovulation. This general concept received support from Sobotta ('14, '17) who with Fischel advocated a muscular theory of egg transport. It has since evidently been generally assumed (Long and Evans, '22) that capsule fluid which appeared immediately prior and disappeared immediately after ovulation found its exit through the oviducts. However, the amount of fluid within the periovarial space in early stage two (Long and Evans, '22) is far greater than that seen within the expanded portion of the ampulla following ovulation. The appearance and disappearance of this fluid may conceivably be accounted for in two ways or a combination of these. First, it may be related to the greatly expanded lymph channels reported for the adnexa at this period (Andersen, '27; see also Wislocki and Dempsey, '39). Second, it may be associated with the capsular opening under consideration.

It is not unreasonable to suppose, as did Sobotta, Fischel, and others, that this capsular fluid serves a real purpose in the safe-conduct of the egg from ovary to uterus. It has been suggested that this service is nutritional in nature; it may be that it serves merely as a medium of support. In any case,

the secretion forms the milieu extérieur of the developing ovum (in fact, of the whole gonad) and as such is of interest. It has been stated (Westman, '26) that the large amount of fluid in the periovarial space during oestrus is either secreted by the ovarial epithelium or sucked in by ciliary action from the peritoneal cavity.

EXPERIMENTAL

To determine whether there is any relationship between the appearance and disappearance of the capsular fluid and the peritoneal opening of the bursa, the following experiment was performed.

Six healthy females averaging about 200 gm. in weight were isolated and the normality of their cycles checked by vaginal smears. Under sodium amytal anaesthesia a laparotomy was performed and the ovary, oviduct, and uterus exposed through a mid-abdominal incision. The peritoneal opening of the bursa was then closed with two sutures of fine single-strand silk thread. In some cases both sides were closed, in others, only one, while on the other a "control" suture was made nearby. At intervals varying from 24 to 96 hours a second laparotomy was performed and the organs in question examined and, in some cases, excised and fixed for histological examination. In all cases an excessive distention of the periovarial sac was evident, the distention being roughly proportional to the time elapsed since the closure of the peritoneal opening (fig. 1). One case (S-IV) may well be reported in detail.

In this case the opening was closed on both sides. Seventy hours later examination disclosed a greatly enlarged capsule filled with fluid. The secretion was typically clear, and the ovary with several enlarged follicles was clearly visible through the stretched capsule wall. The uterus was distended with fluid, and a vaginal smear confirmed the fact that the animal was in proestrus. The abdomen was then closed. Thirty-six hours later, another examination was made. The capsule on both sides showed an even greater distention. It appeared that ovulation had occurred, and the left ovary, oviduct, and uterus were fixed for histological examination.

Although apparently fresh corpora lutea were present, no eggs could be found. It may be of interest to note, however, that in the distended periovarial space several cell-aggregates were found exhibiting a considerable degree of organization (circular masses surrounded by a single layer of flattened cells); these looked very much like products of the cumulus oophorus normally found around recently ovulated ova. It possibly indicates that periovarial fluid may act as a natural "culture medium."

It seemed not improbable that according to either Sobotta's or Fischel's hypothesis of egg transport such a cyst-like distension might readily affect the movement of egg from ovary to oviduct, if not ovulation itself. Therefore exploratory experiments were made on twelve animals. Vaginal smears were made on all animals, and eventually most of them were mated.

Three were treated as follows: one had the capsular opening closed on both sides and two on only one side. The latter came into heat within 48 hours, were mated, and at autopsy a week later showed implantation sites. The one with bilateral closure never came into heat during the 10-day examination period, and at autopsy showed both capsules tremendously extended.

The next four animals were all subjected to bilateral closures and their cycles followed by vaginal smears for a greater time. One animal showed four normal cycles in 16 days; a second showed an oestrous smear only twice in 20 days; the third came into heat three times in 35 days: it was placed with a male for 21 of the 35 days, but at autopsy showed no signs of pregnancy. Both capsules were tremendously enlarged (fig. 1). The fourth animal was placed with a male immediately after operation, and became pregnant. A biopsy on the eighteenth day of pregnancy revealed that foetal absorption was well advanced.

In some instances the ovary appeared on gross examination to have been affected; as a whole it occasionally appeared reduced in size and in one instance seemed to be composed almost wholly of old corpora lutea and a little stroma. The uterus in

some instances appeared enlarged, thin walled, and flaccid and did not exhibit its usual excitability. It is interesting to note that the oviduct did not become excessively filled with fluid. If a constant ciliary current is passing from the periovarial

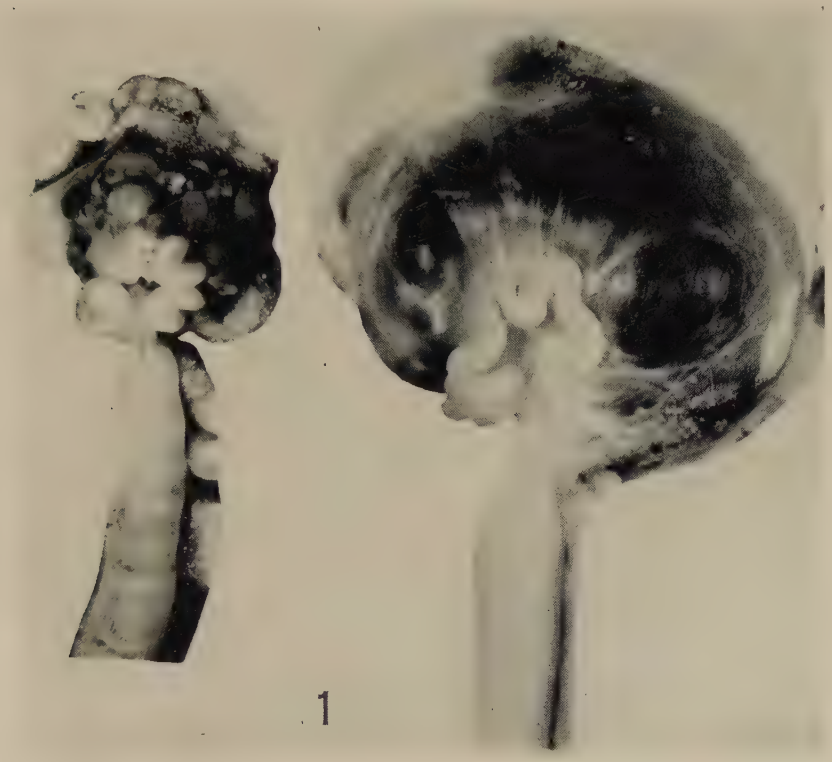

Fig. 1 On the left: ovary, oviduct and cephalic portion of the uterine cornu of a normal animal. Note that the transparent periovarial capsule fits closely about the ovary, visible through the capsule wall, and that the folds of the oviduct lie in a compact group.

On the right: Animal S_1A_4 in which the capsule opening was closed 35 days before autopsy. Note that the capsule is distended with fluid and that the folds of the oviduct are displaced.

space into the ampulla it would be logical to expect that some of the excess fluid would pass into the tube and even expand the second loop, as occurs during oestrus. It seems possible that this latter phenomenon is not the result of a simple

influx of capsular fluid. On the other hand, there may be a constant flow through the tubes into the uterus at times other than oestrus, while at ovulation the tubo-uterine junction may close and the fluid become stored in the tube, the thin walls of the ampullar loops allowing this region to become exceptionally expanded. Possible support for this may lie in the condition found in the uterus, mentioned above.

Six other animals were observed for a still longer period (up to 6 months). A full study of these (and the preceding six) has not been completed, but it may be stated at this time that from one a normal-sized litter was obtained; a second, mated 45 days after operation, showed a nearly full-term litter, to all appearances quite normal. Another animal mated 30 days after the operation showed at autopsy four implantation sites on one side, none (?) on the other. The capsule on this animal measured approximately 1.5 cm. in diameter. An animal mated 6 months after bilateral closure and autopsied 10 days after copulation was especially interesting: neither capsule was fully distended with fluid, but gave unmistakable evidence of having been so, for the capsule was greatly enlarged and instead of fitting closely about the gonad, it appeared oversized and lay collapsed about the organ. The left ovary was markedly reduced in size; the right one appeared less abnormal. The left cornu showed three implantation sites; one very near the utero-tubal junction appeared rather large for its age, the second, immediately below the first, was very much smaller, and the third, near the cervix, was still smaller. The right cornu had four embryos, three near the ovarian end, all closely juxtaposed, and a fourth about two-thirds of the way toward the cervix. Progressing from the upper (ovarian) embryo, each succeeding embryo was smaller than its neighbor, the difference being considerably greater than the variation in size normally occurring.

CONCLUSION

These varied results preclude any generalization concerning the effects produced by the closing of the periovarial

opening. It appears that the ovary-oviduct relationship in the rat is structurally very similar to that reported for the oestrous guinea pig (Sobotta, '17), in which the opening between the peritoneal cavity and the bursa ovarica becomes reduced to a slit, protected by the fimbria of the oviduct. The sometimes considerable effect of closing this slit in the rat suggests that patency is necessary to the normal physiology of the region. That the gonad itself is affected is suggested by its appearance and by the fact that the regular periodicity of the oestrous cycle may be considerably disturbed. Whether this is due to direct action of accumulated fluid or to increased pressure on the ovary is not known. The termination of pregnancy also suggests a disturbance in ovarian secretions. Marked differences in the size of the foetuses possibly indicates that implantation time itself may be altered by the operation. Further, it raises the question as to whether other forms purportedly possessing complete capsules actually do have them, and if so by what mechanism is an excessive accumulation of fluid within the periovarial sac prevented [2]. Any conclusions concerning these and other questions must await further investigation. It is possible that, with modifications, this experiment may throw additional light on the mechanism of ovarian secretion and on the source of the periovarial fluid. It is clear from these results that the periovarial fluid is not sucked into the capsule from the peritoneal cavity, as suggested by Westman ('26).

SUMMARY

1. The periovarial space in the albino rat is not anatomically isolated from the peritoneal cavity but connects with the latter by a slit-like opening on the antimesometrial side of the bursa at the junction of this and the cephalic tip of the uterine cornu.

[2] Since this writing the author has had the opportunity to examine the periovarial sac in two specimens of Mustela sp. The sac is by no means complete — a comparatively large but inconspicuous opening is protected by a greatly expanded infundibulum.

2. The periovarial sac opening is evidently functionally closed at certain periods of the oestrous cycle, most probably by the fimbriated tip of the oviduct.

3. Artificial closure of the opening by suture results in considerable expansion of the capsule, which may persist for long periods. Although it does not prevent egg transport, it may affect oestrous and gestational periods.

4. These results are taken to indicate that the presence of the connection between the bursa and the abdominal cavity is essential to the normal physiology of the region.

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PLATE 1

EXPLANATION OF FIGURE

2 The periovarial sac has been opened by a transverse incision on the antimesometrial side to expose: (a) part of the ovary, (b) the intracapsular portion of the ampulla of the oviduct, just to the left of the hilus of the ovary, and (c) the foramen connecting the periovarial space with the abdominal cavity, entering the capsule at the base of the hilus, below the fimbriated tip of the oviduct. Note that were the lower portion of the capsule to be drawn back over the ovary, to its normal position, the tip of the oviduct would completely or nearly cover the periovarial sac opening.

Inset: External view of the periovarial sac opening. The loops of the oviduct have been slightly displaced to expose the opening.

LISBETH
KRAUSE
2

THE RELATION OF LYMPH VESSELS TO OMENTAL MILK SPOTS

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FIVE FIGURES

An association of milk spots and lymphatics was first suggested by Ranvier (1889) who described clusters of lymphoid cells overlying the lymph vessels in the peritoneal surface of the rabbit diaphragm. He thought the cell clusters marked the site of open communications between the peritoneal cavity and diaphragmatic lymphatics. Since he failed to identify lymph vessels in any portion of the omentum, he did not recognize a similar relationship between milk spots and lymphatics in this organ. Others who support this view are Sappey (1888), Shipley and Cunningham ('16), Seifert ('23), Poynter ('28), and Higgins and Bain ('30). Among those who believed in the existence of omental lymphatics are Haller (1803), Chaussier and Adelon (1815), Luschka (1863), Poirier and Charpy ('01), Heger ('04), Fuller ('08), Norris ('08), Suzuki ('10), Koch ('11), Casparis ('18), Braithwaite ('23), and Marchand ('24). Hamazaki ('25) identified lymph vessels in the omentum and stated specifically that they are present in omental milk spots of dogs, but are quite difficult to recognize in the milk spots of rabbits, cats, and rats. Kampmeier ('28) was of the opinion that mediastinal milk spots are supplied with lymph vessels, and Webb ('31) suggested a similar relationship in the milk spots of the rat omentum. Rostock ('28) and more recently Zschau ('33), Simer ('34 and '35), Dick ('35), Fischer ('36), and Simer and Webb ('37 and '41), demonstrated conclusively the larger lymph vessels of the

omentum. Because he was unable to identify lymphatics in pleural milk spots, Mixter ('41) concluded that all of their vessels belong to the blood vascular system. In this respect he thought that pleural milk spots resemble the spleen.

From the literature cited, it is apparent that the demonstration of lymph vessels in the omentum has been a perplexing problem. Studies of stained serial sections have often been unproductive because it is difficult to distinguish the thin-walled lymphatics from veins, fat cells, and shrinkage spaces. The injection technique has proved to be successful in demonstrating the larger perivascular lymph channels, but is ineffective in delineating their tributaries which are well supplied with valves preventing the retrograde flow of injected fluid. By using the absorption method, Braithwaite revealed large lymphatics in the omentum. These vessels were colored by indigo carmine, absorbed from pads soaked in a solution of the dye and attached to the omental surface. This method seemed to be appropriate for the study of smaller lymph vessels, but our attempts to employ it were unsuccessful. A similar method of coloring mesenteric lymphatics by use of trypan blue is described by Pfuhl ('39) and Pfuhl and Wiegand ('40). Utilizing this dye, we were able to identify the lymphatics of omental milk spots.

Our study was made on the dog, rabbit, rat, and guinea pig. The usual procedure was to inject a 1% solution of trypan blue (Hollborn) into the peritoneal cavity of the animal and, after a time interval, study the omentum. Fresh and fixed spreads and serial sections of tissue were examined for lymph vessels containing the dye. The amount of solution injected and the time it was allowed to remain in the peritoneal cavity varied with the different animals. In the dog, an injection of 10 cc. of dye for 1½ hours was satisfactory; in the rabbit, 8 cc. for 1¼ hours; in the rat, 3 to 5 cc. for ½ hour; and in the guinea pig, 5 cc. for 1½ hours. The material was fixed in Romeis's Susa as recommended by Pfuhl and Wiegand, or in formalin which was equally satisfactory.

Our first attempts to duplicate the experiments of Pfuhl and Wiegand resulted in failure, for none of the mesenteric lymphatics of guinea pigs were colored as they described. Since these investigators did not name the manufacturer of the trypan blue that they employed, we selected Grübler's, a supply of which was on hand. This proved an unfortunate choice, for good results were achieved in later experiments when we substituted Hollborn's dye. The variability in the action of the same dye from different sources shows the importance of giving complete data when recommending its use.

The larger lymph vessels were most evident in fresh omental spreads from the dog. They appeared as undulating, blue lines, shining through the serosa of fat strips. When the spreads were fixed and cleared these lines were identified as lymphatics of the lateral type, connected at intervals with the perivascular plexus. Tributaries of the lateral channels could be traced into thinner portions of the omentum, where they are associated with more deeply stained areas of perivascular fat (fig. 1 A). Study with higher magnification reveals that the lymph vessels of these areas are distended and have a beaded appearance, indicating the location of numerous valves (fig. 1 B). In serial sections of a densely stained area, it is possible to identify the cellular aggregations as adventitial milk spots. These spots are invaded by distended lymph channels, the lumina of which are separated from the cellular elements by only the thin vessel wall (fig. 2). Additional structures which appear in a typical section are blood vessels and scattered fat cells. In these sections, the contrast between lymphatics and other structures is so marked that little difficulty was experienced in their recognition. The presence of trypan blue in most of the lymph vessels permitted their positive identification. In doubtful instances, it was relatively easy to establish a diagnosis by tracing the structures in serial sections.

Fresh spreads of rabbit omentum are similar in appearance to those of the dog, although the thinner portions were more deeply stained by the trypan blue, providing a sharp contrast

with the lighter fat strips. As in the dog, the margins of the fat strips were colored at the locations of adventitial milk spots. It was necessary to use a magnifying lens to observe the course of lateral lymphatics embedded in perivascular fat of the rabbit omentum. In fixed and cleared preparations, branches of these lymph vessels could be traced through the regions of milk spots. Studies of serial sections proved that the relationship between these structures is similar to that in the dog.

Our greatest difficulty was experienced in demonstrating the lymphatics in milk spots of the rat omentum. When trypan blue remained in the abdominal cavity of animals for 1 hour or more, all connective tissues were deeply stained, and the milk spots became opaque, masking the lymph vessels in all omental spreads. Serial sections were of little value because the blood vessels and many fat cells contained as much trypan blue as did the lymphatics. After the action time of the dye had been reduced to 30 minutes, lymph channels were identified in cleared tissue but none were seen in fresh spreads. The lymphatics could be distinguished in serial sections, and small tributaries were traced into adventitial milk spots where they form a plexus.

Attempts to demonstrate omental lymphatics in the guinea pig were successful after the injection of trypan blue. Although no vessels were seen in fresh spreads, they were quite obvious in cleared material. The perivascular plexus was filled with dye (fig. 3) and from it, smaller branches led to deeply stained adventitial milk spots. The identification of these connecting branches is difficult in spreads because they accompany blood vessels, but is easy in serial sections. In figure 4 A, such a lymphatic is shown. The direction of lymph

Fig. 1 Photomicrographs of a cleared spread of dog omentum, 1½ hours after intraperitoneal injection of trypan blue. bv, artery and vein of fat strip; l, lymph vessel of fat strip; ms, adventitial milk spots containing distended lymphatics. Figure A, magnification, × 3. Figure B, higher magnification (× 9) of area outlined in figure A. The beaded appearance of the distended lymph channels indicates the location of valves.

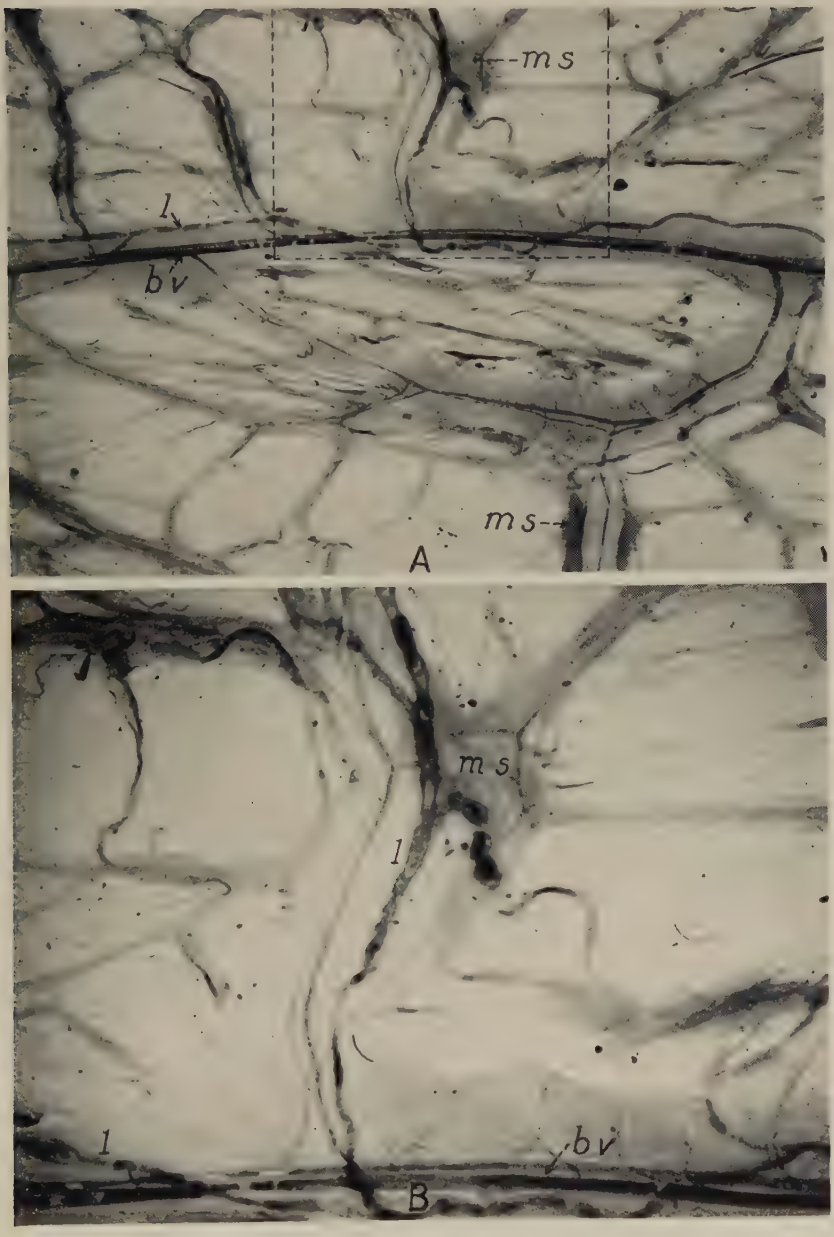

Figure 1

flow from the milk spot toward perivascular channels is indicated by the position of the valve flaps. Accumulated dye in the valve sinuses permits positive identification of this vessel. Tributaries from the lymphatic plexus of an adventitial milk spot unite with the vessel at this level.

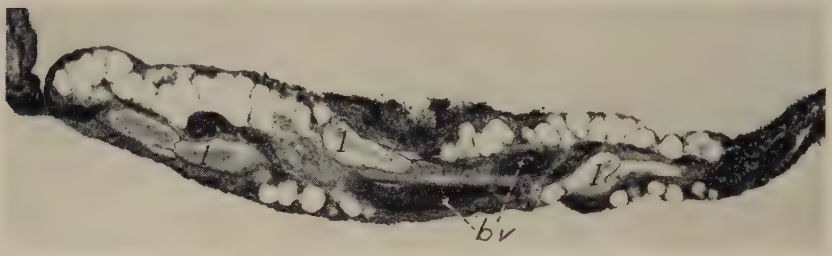

Fig. 2 Photomicrograph of a stained section through an adventitial milk spot in the dog omentum, × 40, 1½ hours after intraperitoneal injection of trypan blue. bv, artery and vein; l, lymph vessels, two of which contain valves.

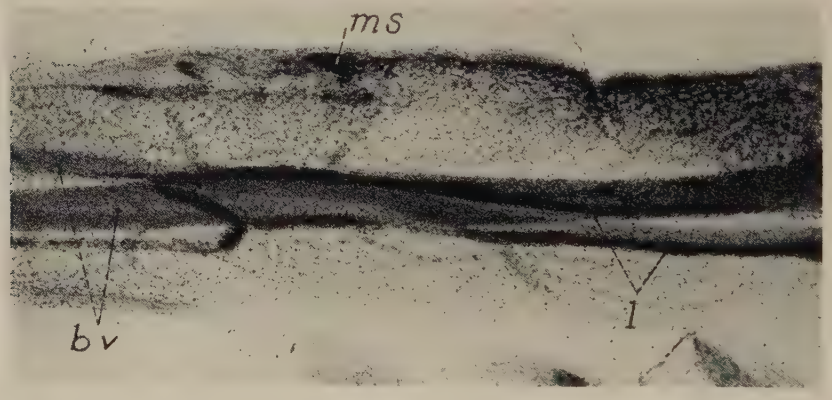

Fig. 3 Photomicrograph of a cleared spread of guinea pig omentum, × 35, 1½ hours after intraperitoneal injection of trypan blue. bv, artery and vein of fat strip; l, perivascular lymphatics; ms, adventitial milk spot.

The relationship of lymphatics to an adventitial milk spot is shown in figure 4 B. Five vessels, whose walls touch the cellular plaques, are present. The largest one, at its junction with a smaller channel, contains a valve, and particles of dye are lodged in its sinuses. The similarity between fat cells and small lymph vessels in this section is so marked that it was

desirable to make a graphic reconstruction of the area to show the connections of these individual channels.

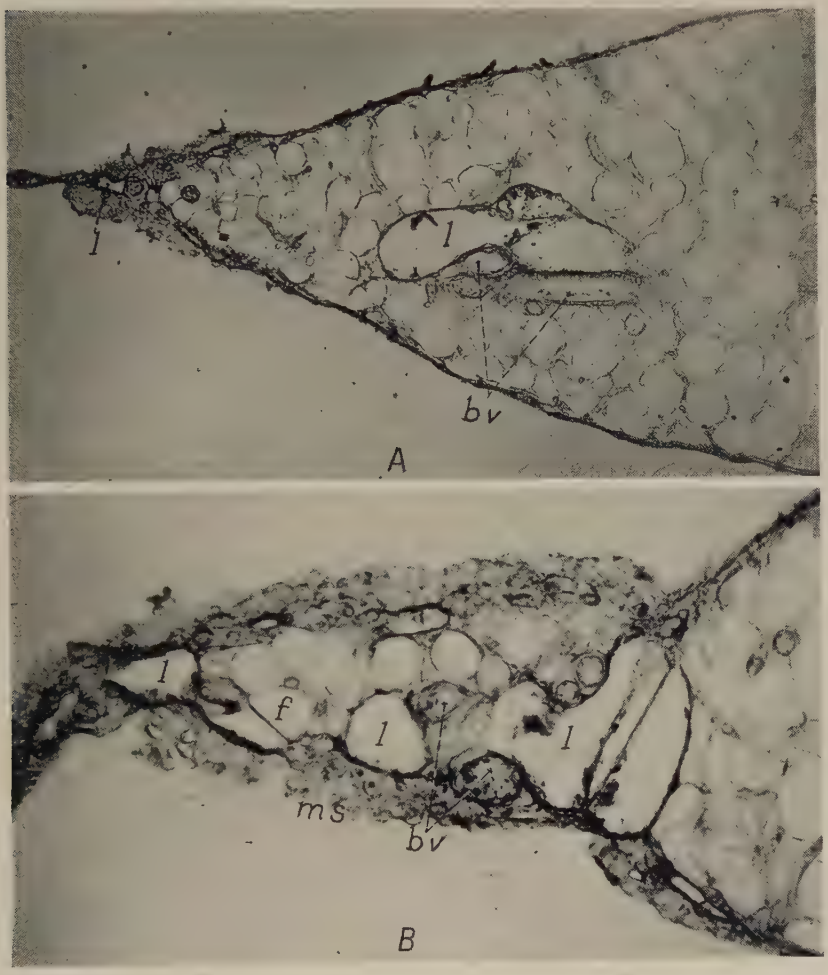

Fig. 4 Photomicrographs of sections of an omental fat strip from a guinea pig, 1½ hours after intraperitoneal injection of trypan blue. bv, blood vessels; l, lymph channels; f, fat cell; ms, adventitial milk spot. Figure A, (× 125), section through the lymph vessel which connects the plexus of a milk spot with perivascular lymphatics. Valve sinuses contain trypan blue. Figure B, (× 235), section through an adventitial milk spot containing a lymphatic plexus. A valve is located in the largest vessel at its junction with a tributary. The planes of section of figures A and B are indicated on figure 5.

A reconstruction of a portion of an omental fat strip from the guinea pig, containing two adventitial milk spots and their

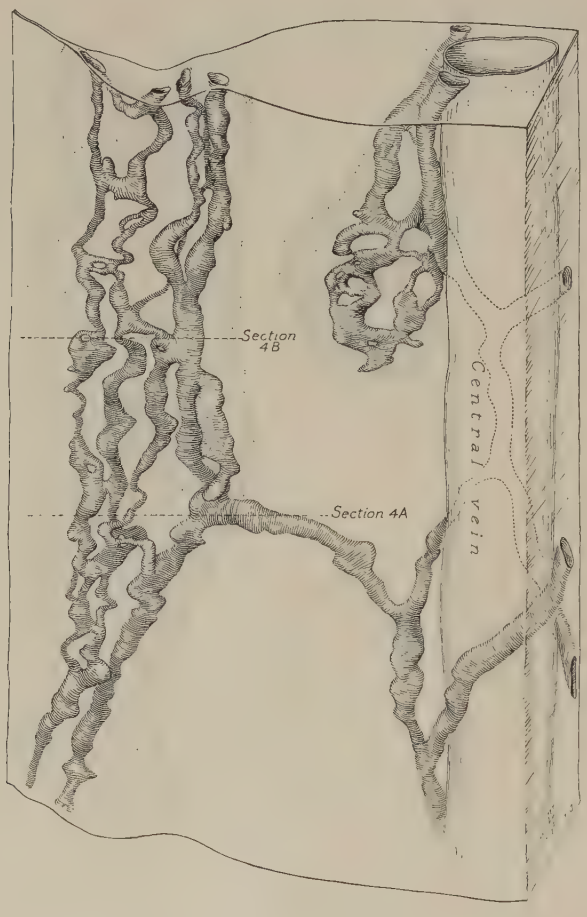

Fig. 5 Graphic reconstruction of a portion of omental fat strip from a guinea pig, × 60. All blood vessels, except the large central vein, are omitted. Lymph vessels are shaded. Positions of two milk spots are shown by stipple. The lymphatic plexus in the lateral milk spot is united to the perivascular lymph channels by a single connecting vessel.

lymphatics, is shown in figure 5. The smaller milk spot is superficial, partially overlying the central vein of the strip,

and the larger one is located laterally, at the junction of the fat strip and the thin part of the omentum. For the sake of simplicity, all blood vessels except the central vein have been omitted. The lymphatics shown in the reconstruction either contained trypan blue or were continuous with such vessels; consequently, several small, doubtful channels are not included. Therefore, the plexiform arrangement of lymphatics in the reconstruction is less complicated than that which exists actually in omental milk spots. The plexus of the lateral spot is united with the perivascular lymphatics by a single connecting channel. Near the margin of the milk spot this vessel is provided with a large valve (fig. 4 A) the flaps of which are directed away from the plexus and, thus, indicate the direction of lymph flow.

SUMMARY

A 1% solution of trypan blue (Hollborn) was injected into the peritoneal cavities of rats, guinea pigs, dogs, and rabbits, following the method of Pfuhl and Wiegand. One-half to 2 hours later, the contents of the omental lymphatics became visible. These vessels appeared as blue lines in the fresh omental fat strips of the dog and rabbit, but were not seen in the guinea pig and rat until the tissue had been cleared. In cleared spreads of all animals it was possible to trace lymphatics into the areas of milk spots, and their intimate relations were observed in serial sections. In the rat and guinea pig, a plexiform arrangement of lymph vessels pervaded the spots. A graphic reconstruction of a small area of an omental fat strip from a guinea pig demonstrates the connections of a plexus to the collecting lymph channels which accompany the larger omental blood vessels. A different pattern was found in the lymphatics associated with milk spots in the dog and rabbit. Here the vessels were larger, less plexiform, and distended in some areas. Our observations confirm the statements of Hamazaki concerning the presence of lymph channels in omental milk spots.

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THE INFLUENCE OF VARIOUS STEROIDS ON THE DEVELOPMENT OF CASTRATION CHANGES IN THE HYPOPHYSIS OF THE RAT

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It is well known that the development of castration changes in the rat pituitary can be prevented by the administration of estrane derivatives with folliculoid [2] activity or androstane derivatives possessing testoid properties. However, the relevant literature contains many puzzling observations. Some investigators (Fluhmann and Kulchar, '31) found "estrin" practically ineffective in this respect, whence they concluded that another ovarian factor must be responsible for the maintenance of the normal pituitary structure in intact females. Other workers (Lehmann, '27; Martins, '31), using rather crude preparations, believed that the "testis hormone" is inactive, or at least only very slightly active, in spayed females although it is effective in castrate males. Similar negative results have been obtained with small doses of androsterone in the spayed female rat (Reece, '41). Conversely it has been claimed that folliculoid preparations are ineffective in the castrate male (Lehmann, '27; Desclin, '34; Yanagita, '37). These observations implied that this effect is, in a sense, sex-specific. Early investigators, who used impure corpus luteum preparations, which were not entirely free

[1] This work was done under the tenure of the Aletta Marty Memorial Scholarship awarded by the Queen's Alumnae Association.

[2] These terms are used here in accordance with the recently proposed terminology of the steroid hormone actions (Selye, '41) instead of the rather clumsy terms "estrogenic" or "follicular hormone-like," "adrenal cortical hormone-like," "corpus luteum hormone-like" or "progestational" and "testis hormone-like" or "androgenic."

of folliculoid activity, concluded that the corpus luteum hormone prevents the development of castration changes at least in the spayed female (Charipper, '34; Clauberg and Breipohl, '34, '35). However, with the exception of Brooksby ('38), all those workers who used pure synthetic progesterone considered it to be ineffective both in male and in female gonadectomized rats (Hohlweg, '35; Migliavacca, '36; Cutuly, '41). Masson et al. ('42) studied the action of three 17-ethyl-etiocholane (pregnane) derivatives on the pituitary of spayed female rats. They found that acetoxy-pregnenolone (21-acetate of 17-ethyl-Δ^5-androstene-3(β),21-diol-20-one) and pregnenolone (17-ethyl-Δ^5-androstene-3(β)-ol-20-one) prevent the development of castration changes, while pregnanedione (17-ethyl-etiocholane-3,20-dione) is ineffective. They emphasized that the first two of these steroids possess other hormonal actions inasmuch as they are both folliculoid and corticoid, while pregnanedione possesses no known hormonal action. These findings, as well as our own observations, led us to suspect that the castration-change-preventing effect of the steroids may be less specific than has hitherto been believed. It has been emphasized by Selye ('42) and Clarke and Selye ('42) that the anesthetic power and the ability to stimulate the vaginal epithelium are pharmacological actions common to all hormonally active steroids irrespective of the specific nature of their main hormonal activity. The experiments to be reported in this communication indicate that the castration-change-preventing effect is also shared by all hormonally active steroids which have so far been tested for this action in adequate doses. Conversely, steroids devoid of any known hormonal effect do not prevent the formation of castration cells.

EXPERIMENTAL

All steroids were administered either in true solution or in fine crystalline suspension depending on their solubility. The relevant experimental details are mentioned in table 1 which is almost self-explanatory. It should be emphasized that not

all these experiments were performed primarily for the study of castration changes. This is why the dosage and the length of treatment is not uniform in all groups. But since the pituitaries were always taken for section, the material is quite satisfactory for the study of the problem under consideration here. The full systematic name of each steroid is given in order to avoid confusion and in those cases in which the compound is generally referred to by a common name, the latter is given in block letters. The melting point of the sample which was available to us for these experiments, was determined in our laboratory and is given in the table as an indicator of the degree of purity of the preparation used. The melting point will also facilitate identification of a compound whenever there is doubt about several possible isomerids.

Perusal of the table clearly indicates that all the hormonally active steroids (compounds 1–12) tested in this series, exhibit the castration-change-preventing effect both in the male and in the female animal. It will be noted that our series includes folliculoid (compounds 1 and 2), testoid (compounds 3, 4, 5, 6, 7, and 12), luteoid (compounds 8 and 12) and corticoid (compounds 9 and 11) substances as well as a steroid whose predominant pharmacological action is to prevent testis atrophy in the hypophysectomized animal (compound 10). For a more detailed review of the literature concerning the hormonal activities of these steroids the reader is referred to the recent article of Selye ('42), but even a cursory study of the chart clearly indicates that the castration-change-preventing action is shared by all hormonally active steroids so far examined. Even though quantitatively some of these may be more active than others, qualitatively the effect is independent of the specific nature of the main hormonal action. Conversely, the hormonally inactive steroids (compounds 13, 14 and 15) are unable to prevent the development of castration changes even if they are chemically very closely related to true hormones. This is clearly shown in the case of pregnanedione (compound 15) which differs from proge-

TABLE 1

Effect of various steroids on the development of castration cells in the anterior pituitary of castrate male and female albino rats.

NO. OF COMPOUND	STEROID	MALE						FEMALE				
		M.P. °C.	Dose in mg./day	Days after castration [2]	No. of animals	Initial body weight in grams	Prevention of castration changes [1]	Dose in mg./day	Days after castration [2]	No. of animals	Initial body weight in grams	Prevention of castration changes [1]
1	$\Delta^{1,3,5}$-estratriene-3,17(α)-diol *-ESTRADIOL*	176	2	11 (0)	5	48	3					
2	$\Delta^{1,3,5}$-estratriene-3-ol-17-one *ESTRONE*	254–256						0.2	13 (2)	3	122	3
3	Δ^{4}-androstene-3-one-17(α)-ol-propionate *TESTOSTERONE PROPIONATE*	118	1	23 (2)	2	160	3					
4	Δ^{4}-androstene-3,17-dione	169–171						4	20 (2)	6	76	3
5	Δ^{5}-androstene-3(β)-17(α)-diol	182–183	2	11 (0)	5	47	2	1	30 (4)	7	82	3
			10	11 (0)	6	46	3					
6	Δ^{5}-androstene-3(β)-ol-17-one *DEHYDRO-ISO-ANDROSTERONE*	137	2	11 (0)	5	47	1					
			10	11 (0)	6	48	3					
7	17-methyl-Δ^{4}-androstene-3-one-17(α)-ol *METHYL TESTOSTERONE*	153	2	11 (0)	4	49	2	4	30 (4)	5	73	3
			10	11 (0)	2	50	3					
8	17-ethyl-Δ^{4}-androstene-3,20-dione *PROGESTERONE*	128	10	11 (0)	6	48	3	15	13 (2)	3	136	2
			10	20 (2)	3	161	3					
			10	21 (0)	2	248	3					

9	17-ethyl-Δ^5-androstene-3,20-dione-21-ol-acetate *DESOXYCORTICOSTERONE ACETATE*	152	10	11 (0)	6	40	2					
10	17-ethyl-Δ^5-androstene-3(β)-ol-20-one *Δ^5-PREGNENOLONE*	186	10	11 (0)	5	47	3	4	30 (4)	4	78	3
								5	33 (10)	3	80	3
11	17-ethyl-Δ^5-androstene-3(β),21-diol-20-one-21-acetate *ACETOXYPREGNENOLONE*	183–184						3	33 (2)	3	60	3
12	17-ethinyl-Δ^4-androstene-3-one-20-ol *ETHINYL TESTOSTERONE*	265–268	2	11 (0)	5	48	2	4	20 (2)	6	75	2
			10	11 (0)	6	46	3					
			3	23 (2)	2	168	2					
13	17-iso-octyl-Δ^5-androstene-3(β)-ol *CHOLESTEROL*	149						4	30 (4)	7	78	0
14	17-iso-decyl-$\Delta^{5,22}$-androstadiene-3(β)-ol *STIGMASTEROL*	168–169	10	11 (0)	6	46	0					
15	17-ethyl-etiocholane-3,20-dione *PREGNANEDIONE*	190	10	11 (0)	5	47	0	3	33 (10)	4	80	0
16	Not treated	....	..	22	1	39	0	..	13	3	129	0
			..	23	6	162	0	..	33	4	80	0
			..	25	11	114	0					

[1] Graded 0–3: 0, indicates no difference between treated and controls; 1, a distinguishable difference; 2, an almost complete absence of castration changes; 3, complete absence of castration changes.

[2] The figures in brackets indicate the number of days which elapse between gonadectomy and the first of the daily injections.

sterone (compound 8) only in that the latter possesses a double bond between C_4 and C_5, yet pregnanedione is quite inactive while progesterone is highly effective in preventing the development of castration cells in the pituitary. It should be emphasized that, unlike the true hormonal activities, anesthetic potency does not necessarily endow a compound with the ability to prevent castration cell formation. This is shown by the same example of pregnanedione which is more potent as an anesthetic than any other steroid enumerated in our table and yet is devoid of the castration cell inhibitory action.

SUMMARY

Fifteen steroid compounds have been studied for their ability to prevent the development of castration cells in the pituitary of gonadectomized rats. It was found that irrespective of their predominant hormonal action, all folliculoid, testoid, corticoid and luteoid compounds, as well as pregnenolone — whose predominant action appears to be to stimulate testis growth — share the ability to inhibit the formation of castration cells in the hypophysis of the male or female gonadectomized rat. The hormonally inactive steroids have no such effect.

It is concluded that judged by the evidence available up to date, the castration-change-preventing effect is a common pharmacological action shared by all hormonally active steroids.

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EXPERIMENTS ON DEVELOPING RATS [1]

III. THE INDUCTION OF ARTIFICIAL PREGNANCY

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ONE FIGURE

INTRODUCTION

The capacity, particularly demonstrated in the rat and mouse, to produce deciduomata has engaged the interest of practically every investigator who has worked upon problems in association with the female reproductive system. The earlier work of Leo Loeb ('07) emphasized the importance of this reaction to the pathologist: it presents a complex which should throw light on specific tissue reactions and is approachable from a great number of experimental angles. As might be expected, a reaction which is obtained so easily and regularly is difficult to analyze. Where, as in this reaction, the agent of stimulation is not specific and where the region responding to stimulation is in a state of constant flux it is usual to find that any single explanation is inadequate and incomplete.

In the years since Loeb's pioneer work, our knowledge of the reproductive mechanism has acquired a few definite points around which we can arrange some of the experimental findings. The work of Fraenkel ('03) showed the functional importance of the corpus luteum which has been confirmed and extended in the researches of Corner and Warren ('19)

[1] See Anat. Rec., vol. 58, p. 31, 1934.

The experiments here reported were completed at the Osborn Zoological Laboratory. A temperory sanctuary for writing was afforded through the kindness of Dr. Davenport Hooker of the University of Pittsburgh School of Medicine, who placed the George Trumbull Ladd Laboratory at my disposal. It is most fitting that this work should be completed in the Elizabeth Steel Magee Hospital where so many periods of labor have successfully culminated.

and Willard Allen ('35) leading to the isolation and later replacement of the action of corpus luteum by its active constituent progesterone. In the same period the interacting potentialities of the ovary and hypophysis were clearly shown by Smith and Engle ('27), and the mechanism of reaction for the rat clearly indicated by Hisaw ('40) and his collaborators. Edgar Allen with Doisy ('24) had shown the importance of the ovarian secretion of theelin, and a few parts of the transitory picture began to fit into the total pattern of reproductive activity. Allen and collaborators have since shown the remarkable reactions of various dosages of theelin upon the cyclic production of changes within the kaleidoscopic uterine mucosa.

The effects of theelin withdrawal and supersaturation give various results and are better studied in primates than in rodents. Fundamentally the reactions should be similar in oestrus and menstruating animals but apparently there is a distinct series of differences not in the end mechanisms but in the intermediate mechanisation. While attempts have been made to homologize the processes these have not been as successful in detail nor are they as simple to correlate as might appear at first sight. The reasons for this are legion, but few of them are obvious.

The fundamental research which made at least the partial understanding of the reproductive mechanism possible is that of Stockard and Papanicolaou ('17) which, in spite of various small inaccuracies, focused attention on the ease with which the cyclic changes might be observed and correlated with the internal changes in the reproductive system. Their biggest contribution was the breaking of the hitherto regarded continuous cycle into a series of studiable fractions and giving the first criteria for the limitations of these fractions. The further refinement of the method by Long and Evans ('22) for the rat cycle and its later application to the mouse by E. Allen ('22) pointed the way to its still wider usefulness in studies in the reproductive field. Today while the students of reproduction may argue keenly about the terminal point

of any one of the fractional parts of the cycle and their rigid limiting criteria there is comparative unanimity with regard to the general laboratory utility of the cycle. Its useful application by the investigator depends upon his judgment and his capacity to modify his end points in the light of his own particular animal and strain. The faults of the limiting criteria for end points in cyclic phases lie totally with the investigator for few of the animals under investigation have ever been adequately instructed in the production of the phases of the cycle although the mechanism of total cyclic condition seems to be genetically indiginous to most of them. The basic picture of occurrences in different phases of the cycle is best only an average reaction from which greater or lesser variations may be expected to occur. The degree of frequency of correlation with the normal average reaction is surprisingly high, particularly when the number of variables is so large as shown by the results experimentally obtained.

The entire reproductive system with its associated primary control in the hypophysis and its secondary controls in the thyroid, adrenal and other complicating organs resembles a balance pivotted with its center upon a ball and socket joint instead of the regular knife edge. In such a system a force applied may be the cause of reaction in any one of a combination of planes. It is in the failure to recognize this type of balance that most of the controversial points arise which tend to cloud the final explanation of the system. In spite of the disregard of the protean nature of this field there are today more points of meeting among the investigators of the reproductive system than there have ever been before. The differences are matters of detail rather than fundamentals and the mosaic while still incomplete represents enough of the total for us to see some of the missing parts as well as to have at least a workable explanation of the total mechanism.

The problem of decidua formation fits into the reproductive problem as a whole for if it is produced experimentally without embryo development the condition of pseudopregnancy results. This is always terminated in the rat in a fraction of

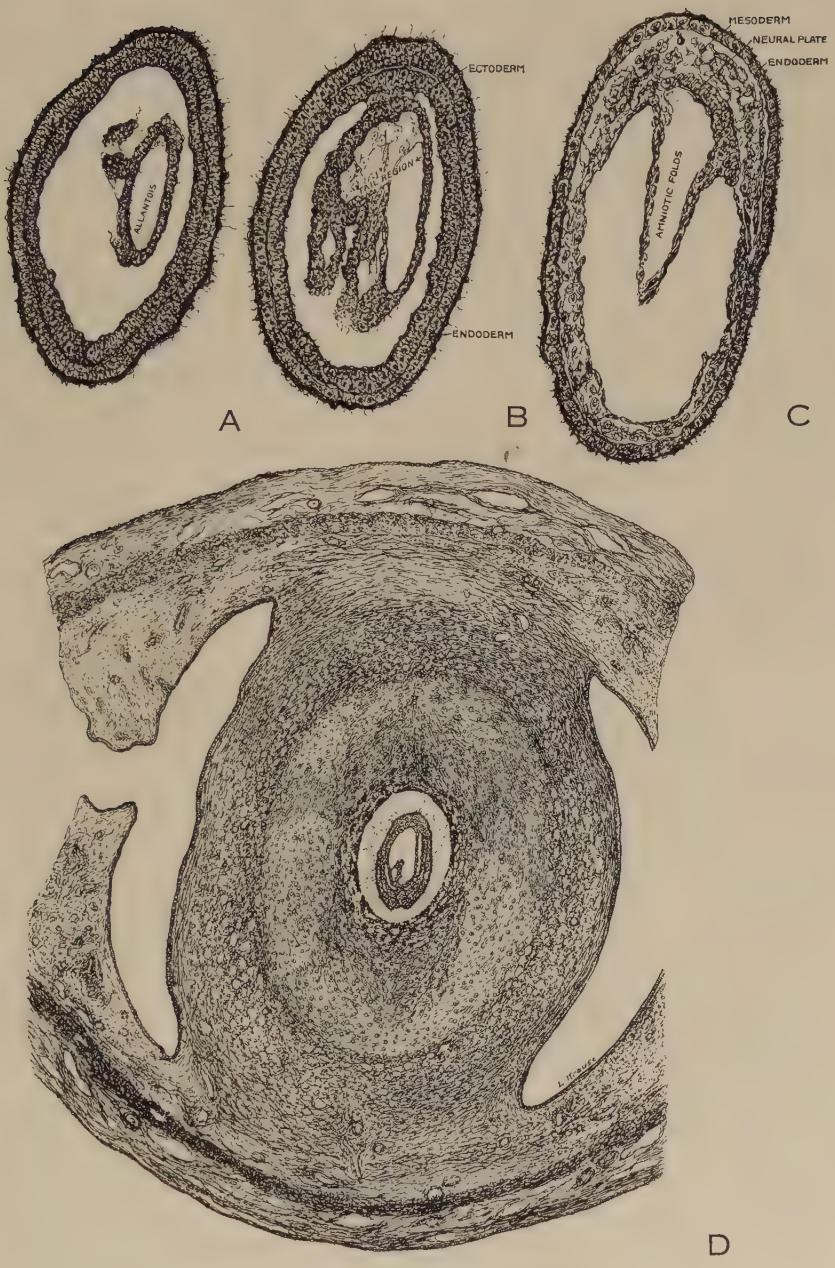
the normal gestation period usually extending through a period of two or three oestrus cycles in length. That the process is linked with oestrus is shown by the relative frequency with which the decidual reaction is obtained at various parts of the oestrus with the same degree of stimulation. As Shelesnyak ('33) has shown, decidual formation can occur in practically any phase of the cycle but occurs with greatest frequency in the periods just before and just after ovulation.

Willard Allen ('31) has shown that the histological picture of experimental decidual formation is identical with that shown during normal pregnancy. This work follows the various reactions within the uterus step by step in such detail that only the one critical step remains to be taken to answer the question of the physiological significance of the reaction with reference to the developing embryo. This the present experiment was planned to do.

EXPERIMENTAL

a. The stage of the cycle at which minimal external stimulation will produce decidual reactions. Long and Evans (l.c.), found that a small glass rod brought within the lips of the vagina was sufficient stimulation to cause skipping of one complete oestrus cycle. This occurred more frequently when the animal was manipulated on the fourth day of the cycle and similar results were secured on the fifth day although here there was a considerable reduction in the number of successful cases. Shelesnyak ('31), however, using electrical stimulation (producing a greater degree of stimulation than Long and Evans) was able to get the same effect at any stage of the cycle and to produce deciduomata even in the

Fig. 1 A, B, and C are sections through the same blastocyst shown in position within the decidua in D. A is a section through the allantoic stalk, B is taken through the junction of the allantois with the embryonic body, while C is taken through the embryonic neural plate. The decidua formed in D is the result of pinching the uterus after vaginal stimulation. The central and marginal stroma show a sharper differentiation from each other than that seen under normal conditions. The mucosal wall shows its normal cryptic components. The differentiation obtained is that of an early 9-day embryo with a slight excess of membrane.



1 ARTIFICIAL PREGNANCY

Figure 1

absence of the ovary. These experiments show that the hypophysis is not acting through the ovary in this specific tissue reaction.

In the cases here reported the stimulation with a glass rod was probably greater in degree than that used by Long and Evans. The results were highly positive as judged by the number of successful deciduomata produced. Stimulation was applied to the vagina rather rigorously in stage three of oestrus.

After stimulation of the vagina, a simple laparotomy was performed and the left uterine horn pinched with fine forceps at five loci approximately equally spaced. Two days later the animal was opened and almost without exception deciduomata were found at the five sites of stimulation. In one case which must have been more than normally sensitized the entire horn was filled with a single deciduoma which showed five incisures at the loci of stimulation.

b. The transplantation of fertile eggs into virgin sensitized uteri. As soon as a sufficient number of cases had shown that the preliminary manipulations produced uniformity of result, the second phase of the experiment was undertaken. This required the removal from fertilized females of the eggs which were secured from the fallopian tubes 24 hours after observed copulation. Thirty eggs were secured in this way and were transferred with a pipette into the left uterine horn of six virgin females prepared as first described. The vagina was further stimulated on the 2 days succeeding the operation.

Several factors, unfavorable to the success of the experiment, were kept in mind; first, massive deciduomata might restrict the distribution of the transplanted eggs and second, the eggs are considerably younger than they normally would be at the time of entering the uterus. The uterus might, therefore, be sensitized for the reception of eggs which of themselves were not ready to meet and implant upon the sensitized areas. This did not prove to be the case, Nicholas ('33) (cf.; Biedl, A., H. Peters and R. Hofstatter, '22).

c. Artificial pregnancy after mechanical stimulation. Development of embryos was secured in four of the six cases; the two negative cases possessed deciduomata but these did not contain embryos. In the four positive females there were seventeen sites in which development either of a fragmentary or total type were secured. Two of these were early 9-day stages, four were retarded 9- or 8-day egg cylinders, six were in 7-day stages while five were abortive or consisted of embryonic fragments with little development or organization. All had progressed sufficiently to show that the initial stages of development had taken place.

The histological examination of this material in general showed that the decidual mass had acted in every case as a suitable surrounding medium for egg development and implantation. In some cases as might be expected, the deciduoma has interfered with the spacing of the embryos and it is probable that in the thirteen negative cases the eggs were completely blocked with no chance for implantation. In none of the four females were all five implantation points occupied. When development was complete the functional decidua presented a different aspect from those in which there were no embryos. This was superficially evident since the decidual mass had a form which was regular and not diffuse; being organized into a pear-shaped mass. This condition may easily checked in section. When the developmental sequence was fragmentary or abortive or where no embryo had developed this was not the case. The deciduomata were larger than in the occupied sites and were also irregularly formed and diffused.

After fixation the uterine horn was cleared and its content examined under the binocular. All deciduomata which showed any signs of embryonic inclusion were sectioned. The functional readjustment of those containing embryonic structures was remarkable for the decidua was well organized, the embryos were properly oriented and the cellular exponents were arranged in regular bands much as in normal conditions of pregnancy.

The oldest embryonic stages secured showed slight morphogenetic irregularities but most of these would fall well within the normal range of variation usually found in development. There is noticeable a tendency for the development of an excess of membrane material at the expense of the embryo within the egg cylinder. This is a condition which has frequently been found in experiments in which the egg cylinders have been cultured in fluid media. It may point to a higher fluid content in the deciduoma.

d. Artificial pregnancy after sensitization with hypophyseal transplants. In this series the animals were mechanically stimulated with a glass rod but the uterine horn was not injured as in the previous series. Each of ten females was given two hypophyseal implants 1 day before the eggs were transplanted. The hypophyses were secured from old males and implanted subcutaneously. After the transplantation of the eggs this treatment was continued. The first four animals of this series were killed 5 days after the operation. They showed degenerating decidua in which no embryos were found. The second pair examined gave one which presented a degenerating small sized decidua and another which was negative. Seven days after the operation one animal of the third pair was negative while the other showed three egg cylinders, one degenerate decidua and one implantation site which had been reduced to a tiny telltale pigmented spot on the antimesometrial border of the uterine horn. The pair examined on the ninth day were negative.

In the light of the findings given above little was expected of the two animals which were alive on the eleventh day but these gave the most positive of all the results in this series. One showed five implantation sites from which the embryos had completely resorbed while the other contained five perfect 12-day embryos. In the effort to carry this case through to term the embryos were lost.

DISCUSSION

The results of these two sets of experiments show clearly that the virgin uterus when properly sensitized can support

all the reactions incident to pregnancy. In the first series where the mechanical conditions which normally lead to pseudopregnancy are fulfilled and subsequently eggs are transplanted to the sensitized horn of the uterus the eggs are to all intents and purposes in a suitable environment for growth and differentiation and further can of themselves organize and mold their surroundings.

The preparatory stimulus obtained by the introduction of a glass rod within the vagina at a slightly earlier stage than that used by Long and Evans (l.c.), is ample to cause the delay of the next cycle. The added imposition of direct uterine stimulation by the five point pinching in addition to a cut made near the uterotubal junction is sufficient to cause deciduoma formation in every case. These are, under optimum conditions, transformed by the developing embryo into a true decidua with its characteristic cellular arrangement.

The spacing of the embryos may be interfered with by excessive damage to the uterine horn in which case the embryos may be bunched and may degenerate. Since the five eggs were introduced simultaneously into the aperture made just below the uterotubal junction this simulated the normal point of entrance into the uterine horn. Their introduction into the tube above the uterotubal junction was attempted but in most cases the eggs were tube locked (compare Burdick, Whiting and Pincus, '37) and did not progress further into the uterus.

In some cases the eggs are forced back through the cut in the uterine wall near the uterotubal junction which undoubtedly accounts for some of the negative results since these never got into contact with the mucosa for attachment. It is known also that there was some egg loss in the pipette for cells and mucosa tend to stick near its orifice at the time of the introduction of the eggs into the uterine horn. In spite of these losses, sufficient cases remain to show that when the egg meets the mechanically stimulated mucosa there is sufficient modifiability of reaction to enable the necessary organization of implantation sites to take place. This is adequate for egg cylinder and embryo formation up to and

including the differentiation of the three germ layers and a complete set of embryonic adnexa.

Krehbiel ('37, '41), on the basis of his histological observations, has concluded that the mucosal reaction produces different types of structure for the embryonic decidua from that formed in deciduomata. It is not within the scope of the present investigation to deny this statement for no close cytological studies were made. The experiments, however, do show plainly that whatever the morphological differences, these have no physiological significance in the course of embryonic development. The ability of the embryo to utilize the crude type of uterine sensitization which normally results in deciduomata formation only and to continue its development within this structure points to a definite adaptability of the embryo to the new set of conditions which physiologically must resemble that occurring normally. The failure of continuance of development rests on other factors than decidual formation. These experiments support Willard Allen's study that the reaction of the mucosa during pseudopregnancy is identical with that of pregnancy. Certainly the minor morphological units which display differences do not inhibit the regular functional phases. The embryo has the capacity for molding the decidua into a definite characteristic form. There is organization in the decidua just as there is in the embryo. This organization is brought about by the embryonic blastocyst and egg cylinder which forms a core around which decidua organization takes place. Whether the embryo or an experimental manipulation serve to initiate the reaction makes no difference in the end result.

The second series of experiments is much more difficult of explanation and fortunately was completed before our knowledge of the hypophyseal mechanism was as extensive as it is today (compare Pincus, '39). It was the normal sequence of another series of experiments in which Antuitrin S was tried as the stimulating agent with uniformly negative result. The hypophyseal material was obtained from old males which

were about to be discarded from the stock of the colony. The grafts were transferred directly from the donor to the host, and were placed under the flank skin of the females. Examination of the site of the transplants showed that the grafts did not take but that the areas in contact with them were hyperemic and the graft was degenerating. All the females used were stimulated vaginally with a glass rod at the time of hypophyseal inoculation. By this method the stimulation of the uterus by pinching was avoided and the eggs were responsible for the creation of their own decidual reaction. The difficulties noted in the first series to embryonic spacing were completely overcome. The eggs responded, developed and were regular.

It is unfortunate that only a hypothetical explanation can be given to the endocrine mechanism behind this reaction but a theoretical deduction from the results is possible. It is now commonly known that there are qualitative as well as quantitative reactions caused by the transplantation of the hypophysis. The vaginal stimulation was sufficient to cause a temporary block in cyclic phenomena which probably is activated through the luteinizing action of the animal's own intact hypophysis. The luteinizing activity so initiated was reinforced by the materials resorbed from the subcutaneously inoculated material resulting in a dominance of the luteinizing phase over the follicle stimulating phase of the ovary.

At the time of embryo inspection there were no evidences of ovulation except in cases in which the ovary was cystic and in which no embryos were found. It is these negative cases which furnish the clue for the explanation given above. The cystic ovaries were hemorrhagic and in some cases contained frank blood, in others a yellow discoloration of the excessively formed follicular fluid. Such findings are occasionally seen in untreated animals and generally are correlated with a failure of embryonic development in the uterine horn attached to such an ovary. It may or may not be effective in limiting the development upon the opposite side. This would account then for those cases in which development did not

ensue following hypophyseal inoculation after mechanical vaginal stimulation. Here the follicle stimulating reaction was dominant either due to insufficient stimulation of the intact hypophysis or to a higher stimulating follicular reaction caused by the male inoculated hypophysis. This is advanced only as a tentative hypophysis of the action of the reproductive mechanism in these cases. It is needless to state that it is subject to radical change with further experimentation along this line. The striking thing is that under such conditions development can and does occur.

SUMMARY

1. Two celled eggs when transplanted to the uterus of virgin rats can develop normally when the uterus has been sensitized by suitable procedure.

2. Adequate sensitization to support development can be secured by vaginal stimulation applied in late stage three of oestrus and, in addition, stimulation either (a) direct, by mechanical methods, or (b) indirect, through hypophyseal transplantation.

3. After the system of direct stimulation has been applied the spacing of the eggs is frequently restricted and many resorptions occur. The developmental sequence is regular in those cases which do develop. Deciduomata are transformed into the typical decidua of development. The implantation sites are directly correlated with the experimental loci of the damage in the uterine horn.

4. Developmental rates are approximately normal. The degree of development attained in the first series was that of a 9-day stage in which the germ layers had differentiated and the embryonic knob was formed. The adnexa sometimes develop at the expense of the embryonic material as is the case in in vitro experimentation with fluid media.

5. When the reproductive system is stimulated vaginally and in addition is responding to two daily hypophyseal transplants development occurred in several cases. The spacing of the embryos was regular, the decidua as regularly formed

in contrast to the preceding series, and in a few cases of advanced development (12-day stages) placental sites were found.

6. The virgin uterus when properly sensitized to produce pseudopregnancy will support development through the early critical stages. Pseudopregnancy is in every respect a limited period of pregnancy both physiologically and histologically.

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